

THE COMPOSITE, HAUSTELLATE MANDIBLES OF
NETWINGED BEETLE AND FIREFLY LARVAE
(COLEOPTERA: LYCIDAE, LAMPYRIDAE)

By

JOSEPH M. CICERO

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TABLE OF CONTENTS

LIST OF FIGURES	iv
ACKNOWLEDGEMENTS	xii
ABSTRACT	xv
CHAPTER 1	
INTRODUCTION	1
CHAPTER 2	
DEVELOPMENT OF THESIS AND LITERATURE REVIEW	10
CHAPTER 3	
METHODS	33
Overview	33
Collection	34
Lycidae	34
Lampyridae	36
Tenebrionidae	36
Processing	38
Fixation	38
Embryos	39
Larvae	42
Dehydration	43
Mounting for SEM	44
Embedment for TEM	44
Mounting on a TEM chuck stub	45
Sectioning	47
Viewing	48
Records	49
Film	50
Artifacts and complications	50
CHAPTER 4	
RESULTS	76
The Larval Lampyrid Syntrophium	77
The mature syntrophium	77
The younger syntrophium	79
The Larval Lycid Syntrophium	80
The mature syntrophium	81
The younger syntrophium	83
The Pharate Larval Lycid Syntrophium	84

The Pharate Tenebrio Larval Mandible	85
CHAPTER 5	
DISCUSSION	157
The Structural Level	158
The base	160
The shank	162
The apex	163
Development	163
Analysis of Hypothesis	164
Anticipation of the Cellular Level	168
Concluding Remarks	169
APPENDIX	
A THOUGHT EXPERIMENT IN HORIZONTAL SALTATION	193
Parasitism and Saprophagy	193
The Experiment	196
The Birthing as a Normal Event	197
Larval Constitution of Lampyridae and	
Lycidae	198
The Birthing as a Freak Event	200
Review of Other Practitioners	201
Developmental	201
Genetic	204
Analysis of Review	206
Developmental	206
Genetic	207
Relevance of the Review to the Birthing . . .	208
Literal atavism	209
Adult Constitution of Lampyridae and Lycidae	212
Concluding Remarks	214
REFERENCE LIST	220
BIOGRAPHICAL SKETCH	228

LIST OF FIGURES

Figure 1. Bearer and one cluster of siblings. Note cannibalism (arrows).	4
Figure 2. The second of two clusters of siblings. . . .	4
Figure 3. Left, one of the sibling larvae. Right, larva of <u>Photuris</u> . These can be compared with a picture of the bearer's head capsule in the appendix (Figure 114, page 216)	6
Figure 4. Current conception of phylogeny of families concerned (Crowson 1972).	6
Figure 5. Apex of sibling abdomen, dorsal view. One of the 4 other siblings bound to it at birth was cut away for dissection. The other 3 fell away from the cord (arrow) that tied them.	8
Figure 6. Ventral view of the apex of a sibling abdomen in strong transmitted light to show light organs (arrow). Note the cord and filamentous pygopod. .	8
Figure 7. Mouthparts of <u>L. sanguineus</u> larva. A) Basal zone. B) Mesal zone. C) distal zone, clearly identifiable as the gala. D and E) Maxillary palpifer. The palpifer is scalloped between these arrows to receive the A-B-C complex. F) Labial palp.	21
Figure 8. Exuvial halves. A) Antenna. B) Ecdysial line. C) Membrane. D) Stylet. E) Labrum, corresponding to (A) in Figure 7. F) Channel from which the stylet can be withdrawn.	23
Figure 9. SEM of <u>Pyractomena</u> mandible, ventral view. A) Acetabulum. B) Basolateral opening to the interior. Mandibles are connected to the head not only by the acetabulum, but also by tissue that passes into this opening.	25
Figure 10. Classical drawings of lampyrid larval mandibles have identified these features. A) Canal. B) Pore. C) Retinaculum. D) Acetabulum. The outer aspect (E), ensheathing the canal, will be referred to as the "cone" in lieu of its actual identity. .	25
Figure 11. Models that might explain channelled mandibles. A) Canal as an apophysis. Inset shows the opposed epicuticles expected if lampyrids conformed to De Marzo and Nilsson's (1986) findings. B) Canal as an apodeme.	27

Figure 12. Mandible of <u>Pyractomena</u> larva longitudinally shaved. A) Basolateral opening, corresponding to Figure 9B.	29
Figure 13. Mandible of <u>Pyractomena</u> transversely shaved. Ventral view.	29
Figure 14. Whole mount of a 1st instar <u>P. collustrans</u> , freed from the embedment plastic after being shaved to the basal half of its mandible.	31
Figure 15. Close-up of mandible in Figure 14.	31
Figure 16. Cross-section of the head of an anatreptic <u>P. collustrans</u> embryo showing the difficulty in identifying which lobe corresponds to which anlage without 3-D reconstruction.	52
Figure 17. Materials used. A) Petri dish with window. B) Dechoriation of egg with two-sided tape. C) Vial rack. D) Eppendorf® vial with tip cut off. E) Beem® capsule modified for minute specimens.	52
Figure 18. <u>P. collustrans</u> embryo embedded and photographed through a compound microscope. See Figure 26. The ideal orientation is shown, with the eyes eclipsed to the viewer.	54
Figure 19. Anatreptic <u>P. collustrans</u> embryo, embedded and photographed through a compound microscope. See Figure 26. A) Mandible anlage. B) Maxillary palp anlage.	54
Figure 20. Cracking the eggshell.	56
Figure 21. Whole embryo affixed to an SEM stub in its native, curled state. The curl is then broken so that the mouthparts face upward.	56
Figure 22. Blebbing of cuticle in an improperly processed specimen.	58
Figure 23. Pharate larval <u>Lycus lecontei</u> , dorsal view, fixed, then removed from its exuviae. Central gap is where stomodeum passes through mouth when the head is pulled out of the exuviae. A) Labromandibular rudiment. B) Gala. C) Maxillary palp.	58
Figure 24. Trial specimen, a mole cricket embryo, dehydrated with HMDS.	60
Figure 25. Well organized SEM stub with all embryos facing the same approximate way along scored lines so that magnification and goniometer adjustment is not necessary for perusal from one specimen to the next.	62
Figure 26. Viewing embedded embryos through a compound microscope. Whittling of the stub was necessary. Figure 18 and Figure 19 were photographed by this means.	64
Figure 27. Sectioning sequence, ultrathin alternating with semithin.	66

Figure 28. SEM stub mounted in a microtome chuck so that tip of specimen's mandibles can be shaved. Large arrow points to fragments of the mandible tip. . .	68
Figure 29. Finder sheet for SEM stub specimens.	70
Figure 30. Blob of glue held between tweezer tines to clean the stub mounted embryo.	72
Figure 31. <i>P. collustrans</i> embryo obscured by adherent yolk and other material.	72
Figure 32. <i>P. collustrans</i> embryo cleaned by the method in Figure 30. Such results are rarely so good as most debris is firmly dried to the embryo. A) Invagination.	74
Figure 33. Lycid mutants. A) Failure to undergo katatrepsis. B) Failure to breach the serosa during katatrepsis. a) Chorion. b) Serosa.	87
Figure 34. <i>Photuris</i> eggs dried to about an 80% loss of spherical volume. Larvae within suffer extreme contortion from strong, localized, pressures. . .	87
Figure 35. First in longitudinal series, from dorsum to venter. Canal actually opens dorsally to the buccal cavity. The section angle makes it appear to open laterally. See Figure 42. A) Dorsal deflection. B) Antenna.	89
Figure 36. Second in long series. A) Amorphous areas. B) Apparent seam is probably a setal socket as no such seam is indicated in transverse sectioning. . . .	89
Figure 37. Third in long series. A) Section grazes retinaculum.	91
Figure 38. Final in long series. A) Section grazes tip. B) Stomodaeal exuviae. C) Base is expanded interolaterally.	91
Figure 39. Close up of amorphous disc in Figure 36A. .	93
Figure 40. First in transverse series of <i>P. collustrans</i> , from base to apex. A) Arrows point to the two lobes that are under observation. B) Antenna. C) Maxillar tissue.	93
Figure 41. Second in transverse series. A) Continuity from transfrontal region to syntrophium.	95
Figure 42. Third in transverse series. Dorsal closure is approached. A) Cells back away from the apex of the 2 lobes to form caps of cuticle.	95
Figure 43. Fourth in transverse series. Dorsal closure of the canal is reached. A) Canal lumen is closed to the mouth at this point.	97
Figure 44. Fifth in transverse series. A) Horseshoe shape of cuticle. B) Area continuous with head tissue is outside the embrace of the horseshoe. .	97
Figure 45. Sixth in transverse section. A) Points to a lateral area that deposits a welt of cuticle of its own between B) and C), the ends of the horseshoe.	99

Figure 46. Final in transverse series. Section cuts tangent to the pore. Below are labial palps, gala and maxillary palpifer.	99
Figure 47. SEM of <u>Photuris</u> 1st instar showing pore, upwardly angled tips and retinaculum, the latter partially hidden by setae.	101
Figure 48. Pore of <u>Pyractomena</u> larval mandible. A) Although partly obscured by foreign material, a seam can be seen to extend apically for a short distance.	101
Figure 49. Long section through the tip of a <u>P. collustrans</u> mandible. Cell diameter is extremely attenuated.	103
Figure 50. Cross-section through extremely tough tissue forming the apex of a <u>P. collustrans</u> mandible. A) Origin of a seta at the end of a dendrite.	103
Figure 51. Innervation of the mandible tip in <u>P. collustrans</u> . A) Dendrite. B) Canal lumen.	105
Figure 52. Tip of <u>P. collustrans</u> mandibles. A) A branched seta at the pore. B) Lack of a seam. C) A campaniform sensilla is on the opposite side.	105
Figure 53. First in series of an anatreptic <u>P. collustrans</u> head capsule. A) Elongate lobe. B) Smaller lobes. C) More lobes occur above (A); these are shown in Figure 54. There is no indication of which lobe corresponds to which mouthpart.	107
Figure 54. Same section as Figure 53. A) Elongate lobe, same as Figure 53A. B) Shorter lobes pointed to in Figure 53C. There is no definitive landmark for orientation.	107
Figure 55. Second in series. A) Section grazes the definitive gala. B) This elongate lobe is the maxillary palp. C) This lobe is therefore the mandible. D) The other lobes are cervical.	109
Figure 56. Final in series. A) The gala protrudes distinctly.	109
Figure 57. Young mandible, prior to commencement of curvature, showing the apical invagination. Also shown by SEM in Figure 32. The 3-armed pattern at the palp apex is a peculiar ripple in the section.	111
Figure 58. Young lampyrid syntrophium at commencement of elongation and curvature. Note the extremely high nuclear: cytoplasmic volume ratio.	111
Figure 59. Young <u>P. collustrans</u> fixed and embedded at a point when the mandibles are first undergoing curvature. A) Stomodaeal exuviae.	113
Figure 60. Long section through the dorsal deflection. A) Incipience of the canal.	113

Figure 61. Facsimile 3-D reconstruction of the <u>P. collustrans</u> mandible. Reconstruction ascertains which of two grazed areas, circles, is of interest. Square shows where Figure 60 is located. A) Incipience of canal. B) Dorsal deflection.	115
Figure 62. Montage of specimen in Figure 60. A) Canal orifice.	117
Figure 63. First in longitudinal series of a <u>P. collustrans</u> katatreptic syntrophium after curvature has commenced. A) Section grazes midlength of the mandible, showing that the canal has progressed at least to this point.	119
Figure 64. Second in long series. A) Origin of the canal. B) Progress of formation is at least to near apex.	119
Figure 65. High magnification of a region in Figure 64, showing the microvilli that appear during canal formation.	121
Figure 66. Third in long series. Section passes beneath the canal and the longest length. A) Conduits are present that are probably haemocoelic. See Figure 67.	121
Figure 67. High magnification of the apex in Figure 66, showing presumed haemocoelic conduits.	123
Figure 68. Head of an unidentified lycid larva. Syntrophia are hypognathous and their bases (A) are contiguous.	125
Figure 69. Interior view of head exuvial halves opened and laid flat as in Figure 8. A) Acetabulum. B) Ridge. See Figure 90. C) Crop.	125
Figure 70. First in an extensive cross-sectional series of <u>Calopteron</u> first instar. A) Labrum is continuous across front. B) Its venter infiltrates the arc-shaped mandible.	127
Figure 71. Second in series. A) Section grazes the anterior extent of the labrum.	127
Figure 72. Third in series. A) Labral halves are separate and, B) fully contained within the arc-shaped mandible.	129
Figure 73. Closeup of the base in Figure 72. A) Nerve bundle is separate from the labral wall at this cross-section. Black spheres are post-stain residue. B) Labromandibular junction.	129
Figure 74. Sectioning continues. A) Gala is reached.	131
Figure 75. Close-up of gala-labromandibular interface.	131
Figure 76. Extreme close-up of Figure 75. Gala is independent.	133
Figure 77. Closeup of nerve bundle in Figure 75. A) Fibrils.	133
Figure 78. Sectioning continues. A) Gala ensheathes. B) Maxillary palpifer appears.	135

Figure 79. Closeup of left syntrophium in Figure 78. A) Center of the mandible. The canal tracks along this longitudinal.	135
Figure 80. Final in series. Closeup of right syntrophium in Figure 78.	137
Figure 81. First in series of <u>Lycus lecontei</u> syntrophium apex. Mandible (lower) and gala (upper) exceed the labrum in length. The gala undergoes dramatic histological change from a cell-filled to, Figure 82, an alveolate interior.	137
Figure 82. Second in series. The alveolate interior of the galar apex is formed by dehiscent spheres and probably functions as a sieve during feeding. . .	139
Figure 83. Final and distal-most cross-section of syntrophium. Mandible exceeds the length of the gala.	139
Figure 84. Exuviae of <u>L. sanguineus</u> stretched over a pinhead so that the mouthparts protrude. Figure 85 through Figure 87 are micrographs of a specimen in this orientation.	141
Figure 85. Apical view, that is, looking down the shank, of the syntrophium. The labral stylet is removed. . .	141
Figure 86. Closeup of oral orifice. A) The mandibles arise as extensions of the pharyngeal wall and, (B), abruptly bend to hypognathly.	143
Figure 87. Close up of the base, diagonal to the mouth orifice. A) The mandible narrow at attachment and, (B), its inner margin rolls over to form a crescent.	143
Figure 88. Anatreptic specimen of <u>Calopteron</u> . A) Mandible anlage.	145
Figure 89. Katatreptic embryo of <u>Calopteron</u> . A) Labrum is a transverse fold on top of the mandible. B) Central flap, perhaps referable to as the hypopharynx. A mote of foreign material is in the mouth.	145
Figure 90. Katatreptic embryo of <u>Calopteron</u> . A) Division between labro-mandibular and maxillar regions. This is the ridge shown in Figure 69.	147
Figure 91. Side view of <u>Calopteron</u> mouthparts showing three layers. A) Labrum. B) Upper mandibular lip. C) Lower mandibular lip. C) Transverse object at apex is the tip broken back by the methods of Figure 28. See Figure 92.	147
Figure 92. Close up of Figure 91. A) Labrum. B) Mandible. Both are appressed to each other as they coextend.	149
Figure 93. Inner ventral view of the <u>Calopteron</u> syntrophium in Figure 91. A) Gala is upwardly concave to receive the labro-mandibular affair. .	149
Figure 94. Esophageal rim of a pharate larval <u>Lycus lecontei</u>	151

Figure 95. Extreme close-up of Figure 94. Mouthpart lobes are positioned along the rim of the mouth. A) Antenna. B) Labromandibular rudiment. C) Maxillar rudiment.	151
Figure 96. Apolysed larva of <u>Tenebrio</u> with exuviae of mandible lifted off like a dental crown to reveal developing mandible within. This can be seen more clearly in the closeup in Figure 97.	153
Figure 97. Close up of Figure 96. A) Pharate mandible with its exuviae removed.	153
Figure 98. Transverse section of pharate larval mandible of <u>Tenebrio molitor</u> showing crenate pattern of cuticle.	155
Figure 99. Close-up of crenation in Figure 98.	155
Figure 100. Attempt to homologize (A) the lycid and (B) the lampyrid syntrophia. Trace of (B) over (A) facilitates comparison, but arrows point only to aspects of (A). a) Inner canal wall. b) canal lumen. c) Outer canal wall. d) Double layers of cuticle.	171
Figure 101. Minimum requirements of lampyrid syntrophial cell identity. A) These must be mandibular. B) These must be labral. C) Dotted line indicates that mandibular cells retreated after laying down cuticle in this area.	173
Figure 102. All cells given identities required to correspond directly to the lycid affair. A) These would be mandibular. B) These would be labral.	173
Figure 103. Close up of <u>Pyractomena</u> mandible shaved as described in Methods. A) Area corresponding to the dotted line in Figure 101 and Figure 102.	175
Figure 104. Summary of areas used in key interpretations for both families. A) Narrow mandibular base. B) Arrows show fluting. C) Lumen. D) Center of mandible. E) Labrum is continuous with transfrontal tissue. F) Labrum undergoes fluting. G) Welt of cuticle.	177
Figure 105. Typical textbook illustration of a mandible. No indications are made regarding where the cells filling the interior (cut-away) originate from.	179
Figure 106. Tracking of the lampyrid canal.	181
Figure 107. A) Lycidae. Labrum (a) falls short of mandible (b). (c) Alveolate gala. (d) Tip of mandible, cut for viewing path of imbibition through pore. B) Lampyridae. Pore is formed because labral field (a) is shorter than mandibular field (b).	183
Figure 108. An interpretation of the invagination in Figure 32A. A) Mandible anlage flanked with labral cells. B) Labral cells infiltrate. C) Invagination is cast off. D) Anlage is smooth again. E) No apodeme was found.	185

Figure 109. Cross-section of anlage in Figure 108 held static while cell fields pass through during development. A) Flank of labral cells. B) Invagination. C) Infiltration. D) Fluting. E) and F) Separation.	187
Figure 110. Mesal section of a 1st instar <u>P. collustrans</u> syntrophium. A, B and C show three directions from which cuticle might be deposited. A) Labrum, B) upper mandibular lip, C) lower mandibular lip. . .	189
Figure 111. Close up of the base of the lycid syntrophium in Figure 73B. A) Mandible. B) Labrum. C) Labromandibular junction. The junction need only be displaced outward, in the direction of the arrow, for the two anlagen to be made one.	189
Figure 112. Generalized beetle head capsule overlaid with a compartmentation pattern that might be expected from what we know of <u>Drosophila</u> cell behavior.	191
Figure 113. One of numerous vesicles composing the pygopod of lampyrid larvae.	216
Figure 114. Head of <u>L. sanguineus</u> larva, the bearer in figure 1.	216
Figure 115. Abdominal apex of <u>L. sanguineus</u> larva. The pygopod is probably the protracted rectum, shown here in its retracted position.	218

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By

Joseph M. Cicero

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During netwinged beetle embryogenesis, the labrum and the mandible develop as separate anlagen. The anterolateral corners of the labrum grow out as stylets. They are positioned on top of the mandibles during this elongation, and their inner faces take on a crescentic fluting. The mandibles grow out as stylets and undergo fluting too, but, in contrast, the crescent is open to the lateral aspect, rather than to the inside. These two then interlock, with the labral stylet inside the flute of the mandibular stylet. This arrangement creates a food canal: the inner face of the labral stylet becomes the canal's outer wall, while the outer face of the mandibular stylet becomes the inner wall.

During firefly embryogenesis, only one anlage develops in the position expected for labrum and mandible. This lobe elongates, curves, attenuates to a sharp tip, and its axial

cells pull away from each other to form a canal lumen. The entire sucking apparatus forms without cuticle-bound intermediates.

By comparing developmental aspects of the two families it is suggested that the labral and mandibular anlagen of fireflies have somehow united, so that cells of each migrate out to positions required of them to form the mature structure first, then lay down cuticle as a finishing touch, bypassing developmental intermediate stages.

This study was prompted by the discovery of a netwinged beetle larva that had ostensibly given birth to firefly larvae. It seemed that a logical first step would be to study an aspect of basic biology that might lead to a better understanding of the relationships between them. Mouthparts were chosen because they serve as important diagnostic characters that separate the two families taxonomically.

Possibilities on how such a birthing could be so fall into two groups. If the sibling firefly larvae are not a product of the bearer's genome, then parasitism and saprophagy are likely causes. If, however, the siblings were born by paedogenesis, then horizontal saltation can be invoked. Horizontal saltation is discussed as a thought experiment that explores such possibilities as hypermetamorphosis, chromosome rearrangement, and change in the regulatory system that controls development.

CHAPTER 1 INTRODUCTION

This investigation was prompted by an extraordinary discovery made in May of 1981 by Robert J. Weich in Tucson, Arizona. At Campbell and Table Mountain Roads, he collected a 30mm by 9mm larva of Lycus (Neolycus) sanguineus (Gorham) (Lycidae) from which, after being dropped in preservative, issued 9 larvae through a rupture in abdominal intersegment 3-4 (Figure 1, Figure 2). These larvae are of the related family Lampyridae (Figure 3, Figure 4), but nothing in the known biology of either family suggests how such a relationship could come about. The host or mother, it is not absolutely certain which, will be referred to as the "bearer."

The bearer's internal tissue had been consumed and some sibling cannibalism occurred, accounting for the considerable development, to 14mm by 3mm, of the survivors. Gonads of undetermined sex are present. Tanning and pigmentation had occurred. A cord of some sort, issuing from within the anal end of each sibling (Figure 5, Figure 6), had knotted them together by their anal prolegs into 2 groups of 5. Tissue masses corresponding in shape, position, cellular structure and tracheal attachment to light organs of larval Photuris are present, as well as fenestrations which, in Photuris, allow

light to emanate (Peterson 1970). Neither ability nor inability to luminesce could be observed.

I would not expect anyone to believe such a report until it is somehow verified. Disappointingly, however, there are several reasons to think that determination of the mechanism involved will come with great difficulty, if ever at all. The birthing is so extraordinary as to render for consideration only the most imaginative of conceivable mechanisms. Parasitism, or scavenging, by a species with or without adults, paedogenesis resulting in horizontal saltation or atavism, horizontal gene flow (Dawkins 1982:159), perhaps others, are so far-fetched that even collection and observation of another such birthing would constitute no evidence in favor of one or the other. A further complication is that larvae of L. sanguineus are very difficult to locate in the field and rear in the laboratory for the controlled experiments required to determine the source of the genes at play, whether intrinsic or extrinsic.

On the practical side, this discovery has provoked two lines of meaningful and productive research. The first is appended to this dissertation. It is a thought experiment that accepts as its initial conditions the possibility that the siblings are a product of the bearer's genome, and invokes horizontal saltation as the explanation. The second line developed from the idea that the main focus of attention might best be placed on developmental morphology. So little is known

about this aspect of the families concerned that a careful examination of key character ontogeny would be a logical first step toward an understanding of how this birthing could be so.

The mouthparts, and in particular, the mandibles are of extraordinary design and are used as main diagnostic features. Because of these two aspects, I have decided to dedicate this dissertation to their elucidation.

Figure 1. Bearer and one cluster of siblings. Note cannibalism (arrows).

Figure 2. The second of two clusters of siblings.



Figure 3. Left, one of the sibling larvae. Right, larva of Photuris. These can be compared with a picture of the bearer's head capsule in the appendix (Figure 114, page 215)

Figure 4. Current conception of phylogeny of families concerned (Crowson 1972).



Telegeusidae
Phengodidae
Lycidae
Lampyridae

Figure 5. Apex of sibling abdomen, dorsal view. One of the 4 other siblings bound to it at birth was cut away for dissection. The other 3 fell away from the cord (arrow) that tied them.

Figure 6. Ventral view of the apex of a sibling abdomen in strong transmitted light to show light organs (arrow). Note the cord and filamentous pygopod.



CHAPTER 2 DEVELOPMENT OF THESIS AND LITERATURE REVIEW

Withycombe (1924) showed a remarkable curiosity in recognizing that lycid larvae possess a paired, tubular, piercing and sucking organ composed of three components.

...the mandibles are rather short and sharply pointed, grooved dorsally. Upon each, dorsally, lies a pointed 'prostheca' or 'lacinia mobilis', grooved ventrally, so that the two appendages form conjointly a tubular piercing and sucking organ very similar to that formed in Neuroptera by the mandible and maxilla. The maxilla lobe (? lacinia) is short and tapering, but blunt-ended. It is also grooved, and is in life often applied to the mandible. There is a short, four-jointed maxillary palpus, and a shorter, three-jointed labial palpus...(page 322)

Scanning electron microscopy (SEM) shows that three zones are indeed involved (Figure 7). The basal and distal zones are rugose, setose, and separated by a smooth, glabrous mesal zone that appears to have a longitudinal seam. Simple probing with a dissecting pin shows that the distal zone is actually the galea that has undergone internal collapse so as to allow it to ensheath the mesal zone. Further, by pulling the dorsal and ventral halves of the head exuviae apart (Figure 8) and laying them flat, it is seen that the basal zone, the 'prostheca', is separate from the mesal zone and might actually be the labrum. Labrum and clypeus are considered absent in larvae of this family (Crowson 1955). The modern and foremost interpretation,

that the mandible is not composite, but instead cleft to base into two lobes (Lawrence 1991), is incorrect.

Scanning electron microscopy of the larval lampyrid mandible shows nothing in common with this arrangement (Figure 9). In fact, the only shared feature is symmetry--there is no left- or right-handedness as in certain other families (Hillerton 1986). No aspect of the head is associated with it except two tissue-connecting sites (Figure 9B, Figure 12).

Returning to the head exuviae of Lycidae, close inspection shows that the mesal zone is actually hollow and a thin stylet can be lifted out from inside (Figure 8D). Still further, the basal segment of the maxillary palp (the maxillary palpifer) abuts closely and takes on a scalloping that ensheathes the 3-zone complex in the area of contact (Figure 7D,E).

The larval lampyrid mandible was known to be channelled early in this century (Haddon 1915, Vogel 1915) (Figure 10), and one cross-section of the cuticular ultrastructure has been published (De Marzo and Nilsson 1986). These authors were studying the mandibular channel of larval dytiscids and looked to the channel of Lampyris noctiluca (L.) for any possible similarities. They found that it, and the channels of dytiscid genera considered highly evolved, have a seam. They refer to the seam as a "fused area", and believe that it represents the

interface between upper and lower lips of a longitudinal groove (Figure 11).

Their belief is based on the pattern by which cuticle is laid down, and on an assay referable to as "intermediate character state comparison." The pattern is of two cuticles with their epicuticles closely opposed to each other (Figure 11, inset) suggesting an invagination. The character states are of other dytiscid larvae, which show a gradation from an open groove to a closed one. An initial inspection of the channel in lampyrid species available to me shows that they are at least superficially consistent with De Marzo and Nilsson's cross-section of L. noctiluca (Figure 12 through Figure 15). The inspection involves embedding the mandible in plastic and sectioning through half its length or diameter. The remaining half is then freed from the plastic with a powerful oxidizing agent (see Methods) and coated for SEM. It is clear that cuticle does indeed line the lumen of the canal as well as the outside as one would expect, but the fused area is not apparent.

De Marzo and Nilsson's presentation of concordance between this gradation and gradations of other dytiscid characters makes for a tight case that satisfies contemporary phylogenetic systematists. However, the composite nature of lycid mandibles, together with the relatedness of the two families, as well as the lack of a fused area, are grounds for investigating the lampyrid mandible further.

There are at least 2 models that satisfy the De Marzo cross-section (Figure 11). In one, the canal is a groove and in the other, it is an apodeme which starts either apically, basally or perhaps even internally. Both are common endoskeletal features, except that apodemes do not penetrate the opposite end to produce a continuous path. A more appropriate reference would be hollow tentorium. Either model as well as others are possible, but verifying which may not determine whether we are dealing with two structures, one (the canal) internal to the other. Might the mandible be inside the labrum, or the labrum inside the mandible? A terminological problem arises immediately which can cause confusion in this dissertation. Therefore, during the descriptive phase, the aspect that the canal is housed in will be referred to as the "cone" (Figure 10).

The gala of lampyrids is involved in a maxillo-labial affair that functions to scoop up food (Cicero 1982:275, fig.7E; unpublished observations) and is independent of mouthparts dorsal to the buccal cavity. However, there still could be a relationship between the mouthpart complexes in these two families. An investigation of their actual construction is in order, and will be the specific mission of this dissertation.

To determine actual construction, it will be necessary to track the mouthparts during embryogenesis and possibly also during pharate larval development. Regarding the latter, the

question of how mouthparts of larval instar X+1 are reconstructed from larval instar X has never been considered. The epidermal cells in question may retain the original shape by using the exuviae as a template or they may retreat back to the head cavity and then reconstruct the mandible anew. Such rebuilding may be a recapitulation of embryonic processes, or a different program altogether. The cells might also die back to stem cells which, in turn, would regenerate daughter cells to reassemble the mandible. In any case, the components involved might be separate and distinct at that time, within the hidden confines of the exuviae.

A small literature exists for mandibles of larvae in other beetle families. It is primarily composed of economic species, such as stored product pests, and it is focused on ways to identify species whose fragments reach the market as contamination (Kuenberg 1977, 1981). Because of the variety of shapes and dentations, as well as their durability, mandibles are usually isolated in an intact state and can be matched up to their owner in an atlas.

Necrobia rufipes (Cleridae) is perhaps the most closely related stored pest to cantharoids, but our knowledge of its mandible offers no obvious correlations. It possesses a ridge on the ventral surface of the mandible that extends from the molar area to the tip of the incisor, giving the appearance that the mandible has upper and lower halves. The labrum of clerids is described as free and distinct (Lawrence 1991).

Mandibular morphology, asymmetry and differential wear are popular ecological assays for determining feeding habits (Smith and Sears 1982; Wallin 1988), and can be used in conjunction with midgut content surveys. These papers use the conventional repertoire of terminology (Peterson 1982), recognizing the uni- bi- and tripartite incisor, mola, prostheca, penicillus, retinaculum, condyle, acetabulum, lateral and cutting edges. They certainly had no reason to venture the slightest suspicion of composite construction.

My challenge of the view that the "mandible" is a singular unit in serial homology of mouthparts is a totally novel pursuit for Coleoptera. Major primary literature, such as Snodgrass (1935, 1951), Matsuda (1965) and Manton (1977) as well as secondary literature (i.e. Chapman 1971) are uniform in their presentation of the mandible as a discrete, homological unit throughout the phylogeny of Mandibulata.

The primary literature offers very little in the way of mandible anatomy also. Zacharuk (1979) has ventured into the histological diversity of larval mandibles, i.e., those of tanned and teneral larvae as well as their exuviae, but from standpoint of seeing how cuticular hardness and mechanical advantage develops with each molt. Similarly, Hillerton's team (1984) found that hardness was gained by inclusion of zinc and manganese to the extent of several percent dry weight of the mandible in some beetles.

No other relevant information was found in the literature on Coleoptera. It seems that if any preparatory help is to come from prior works, it should be found among classically haustellate orders. There might at least be features unique to composite mouthpart design.

The term syntrophium was coined by Jobling (1976) to replace the imprecise term "fascicle" used by earlier authors for the piercing organ of Diptera. He does not state the rationale by which he decides which stylet belongs to which primitive structure. Aphid stylets were given designations in 1928, but to investigate any earlier than 1976 would be futile as it predates the coming of age of cytology and developmental biology. Such designations are carried forward to the present and are assumed by their basal orientation to be correct. It can be argued that these designations need to be rechecked since the stylets are minute, closely appressed, and their bases are protracted (i.e., Wiesenborn and Morse 1988).

In Drosophila, the mouthparts have been traced back to the original compartment they arise from. Compartments are perhaps the ultimate designation. They refer to the minimum number of cells, called polyclones, which are generated from the same parental cell and which minimally embrace the structure in question (Garcia-Bellido et. al 1973).

A checklist of things likely to be found in haustellate mouthparts can bring this chapter to an end.

Salivary canal. In aphids (Forbes 1969) it is unpaired and formed by an internal ridge of the maxillary stylet that rolls over onto itself. In psyllids (Forbes 1972), it is paired and formed from hollows in the interlock that holds the maxillary stylets together.

Food canal. This duct is formed by the space between the interlocked maxillary stylets and open to the outside. Modern investigations, with their conviction to demonstrating actual function, have gone to the extent of fixing the beak while it is embedded in plant tissue, and sectioning for the processes that are involved in imbibition (Kimmins 1986; Spiller 1990).

Unity. Stylets must be held together somehow, so that they may be deployed as a unit. Maxillary stylets interlock mechanically, like jigsaw puzzle pieces, which keeps them appressed against each other, combines their tensile strength, and allows them to slide upon each other as the need for bending the syntrophium arises. Mandibular stylets concavely embrace them but do not interlock. There is no information on how they are prevented from bowing out apically, where they are distant to the labral or other sheaths. Forbes (1969) mentions that coadaptation of contour between mandible and maxilla holds them appressed mechanically and helps to keep stylet bundle compact, but inspection of his own photos shows only a weak co-sinuate contour that is not convincing in this regard. When dissected free of each other, both mandibular and maxillary stylets are bowed inwardly. Possibly the cuticle is

laid down such that the laminar infrastructure always maintains an inward convexity. Air space occurs between maxilla and mandible but this has not been traced to see if interlocks occur periodically. The air space may normally be filled with saliva, water or other liquid, which would provide capillary action that keeps the stylets appressed. However, in midges (Jobling 1976) mentions mandibular base levers and adductor-abductor muscles with no specific address to their possible involvement in alignment.

Innervation. The discovery of neural tubes in the stylets (Forbes 1966, 1969, 1972, 1977 and references) fit well into the climate of scientific investigations of the time, wherein questions on the physiological mechanism of behaviors such as extension, positioning, penetration, inoculation, taste and extraoral digestion were forefront in the agricultural theater (i.e. Spiller 1990). These tubes are referred to as dendrites instead of axons, because branching can be detected by counting their cross-sections at various levels in the length of the syntrophium. Axons do not branch.

Dendrite branches can be found free in the sheath or imbedded in cuticle where they presumably service external sensory organs. Forbes was not able to locate dendrite-organ junctions; however, Honomichl (1978) and Zacharuk (1979), respectively, found proprioceptors and scolopophorous organs in their study animals. The actual locations of the cell bodies have not been established.

The tubes are filled with a clear fluid, contain neurotubules, and are enclosed in an electron opaque cuticular sheath. Pollard (1972) presumed the fluid to be haemolymph and the absence of haemocytes to be due to the impassable diameter. Fibrillar tubular bodies occur outside the sheath (Forbes 1972:565). Glia are not mentioned by the authors and not apparent in their micrographs. So-called multivesicular bodies have been found, and these are now well-known as neurohemal vesicles.

Cibarium. This is a food chamber that is variously formed depending on which structures wall off the mouth during imbibition of food. It is pulsatile in some insects (i.e. Hemiptera) but not in others (i.e. Orthoptera) (Snodgrass 1935).

Sitophore. The sitophore is defined by Snodgrass (1951) as an expansion of the hypopharynx that makes up the floor of the cibarium. Troughlike in shape, it serves to hold masticated food before delivery to the mouth. He illustrates it for larval Dytiscus (loc. cit., p. 103, fig. 37G) as a transverse rod that spans the gap between the mandibles, but doesn't indicate that it is involved in their anatomy or function.

Miscellaneous. An amorphous zone, circular in cross-section, appears at the base of the aphid maxillary stylet in Forbes' (1966) micrograph, but he doesn't identify or comment on it.

Adelgid stylets are curious in that they are studied by embedding and sectioning the whole body (Forbes and Mullick 1970). Sections pass through the stylet bundle as many as 8 times.

Fine pictures of nematode stylet ultrastructure are available but not applicable to insects. Stylets are regenerated from arcade-like progenitor cells during molting (Endo 1985).

Figure 7. Mouthparts of L. sanguineus larva. A) Basal zone. B) Mesal zone. C) distal zone, clearly identifiable as the gala. D and E) Maxillary palpifer. The palpifer is scalloped between these arrows to receive the A-B-C complex. F) Labial palp.



Figure 8. Exuvial halves. A) Antenna. B) Ecdysial line. C) Membrane. D) Stylet. E) Labrum, corresponding to (A) in Figure 7. F) Channel from which the stylet can be withdrawn.

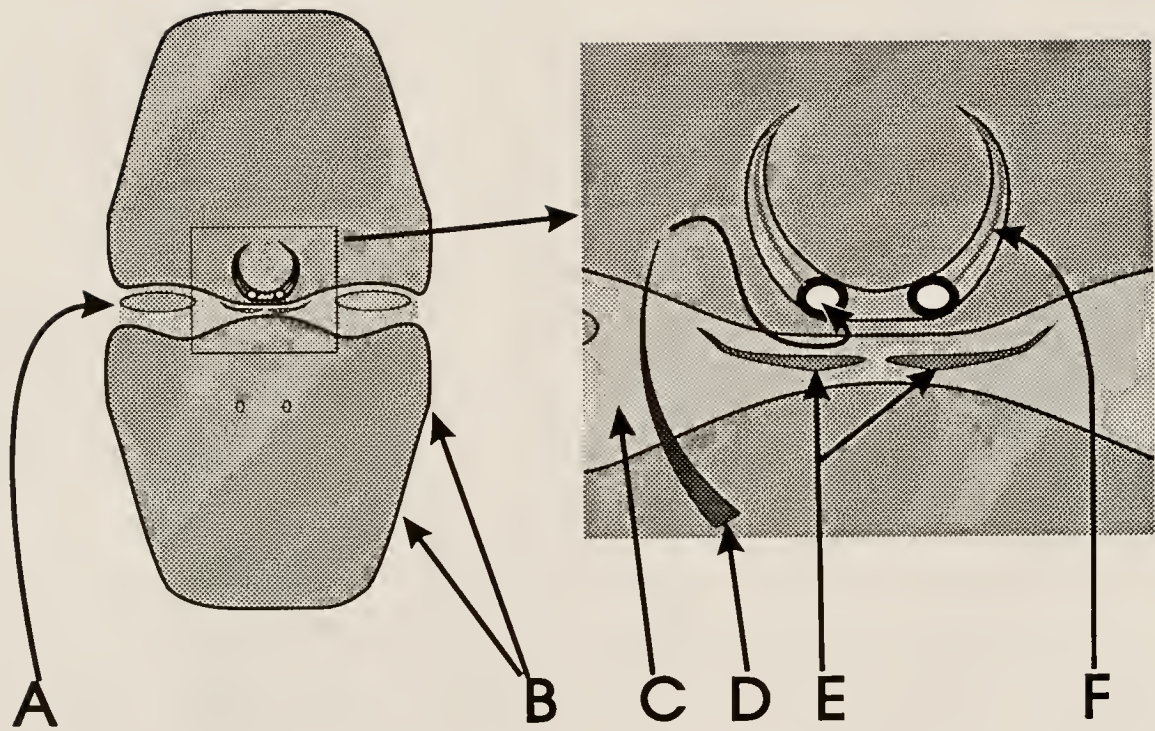


Figure 9. SEM of Pyractomena mandible, ventral view. A) Acetabulum. B) Basolateral opening to the interior. Mandibles are connected to the head not only by the acetabulum, but also by tissue that passes into this opening.

Figure 10. Classical drawings of lampyrid larval mandibles have identified these features. A) Acetabulum. B) Canal. C) Pore. D) Retinaculum. The outer aspect (E), ensheathing the canal, will be referred to as the "cone" in lieu of its actual identity.

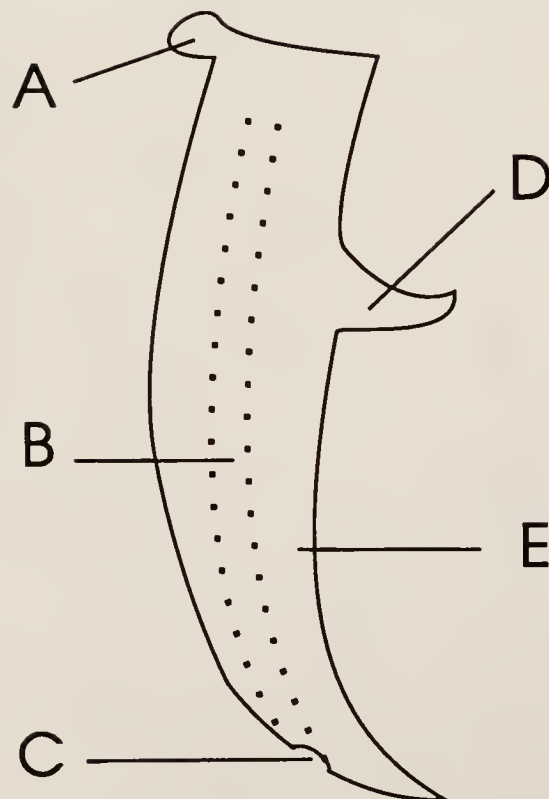


Figure 11. Models that might explain channelled mandibles. A) Canal as an apophysis. Inset shows the opposed epicuticles expected if lampyrids conformed to De Marzo and Nilsson's (1986) findings. B) Canal as an apodeme.

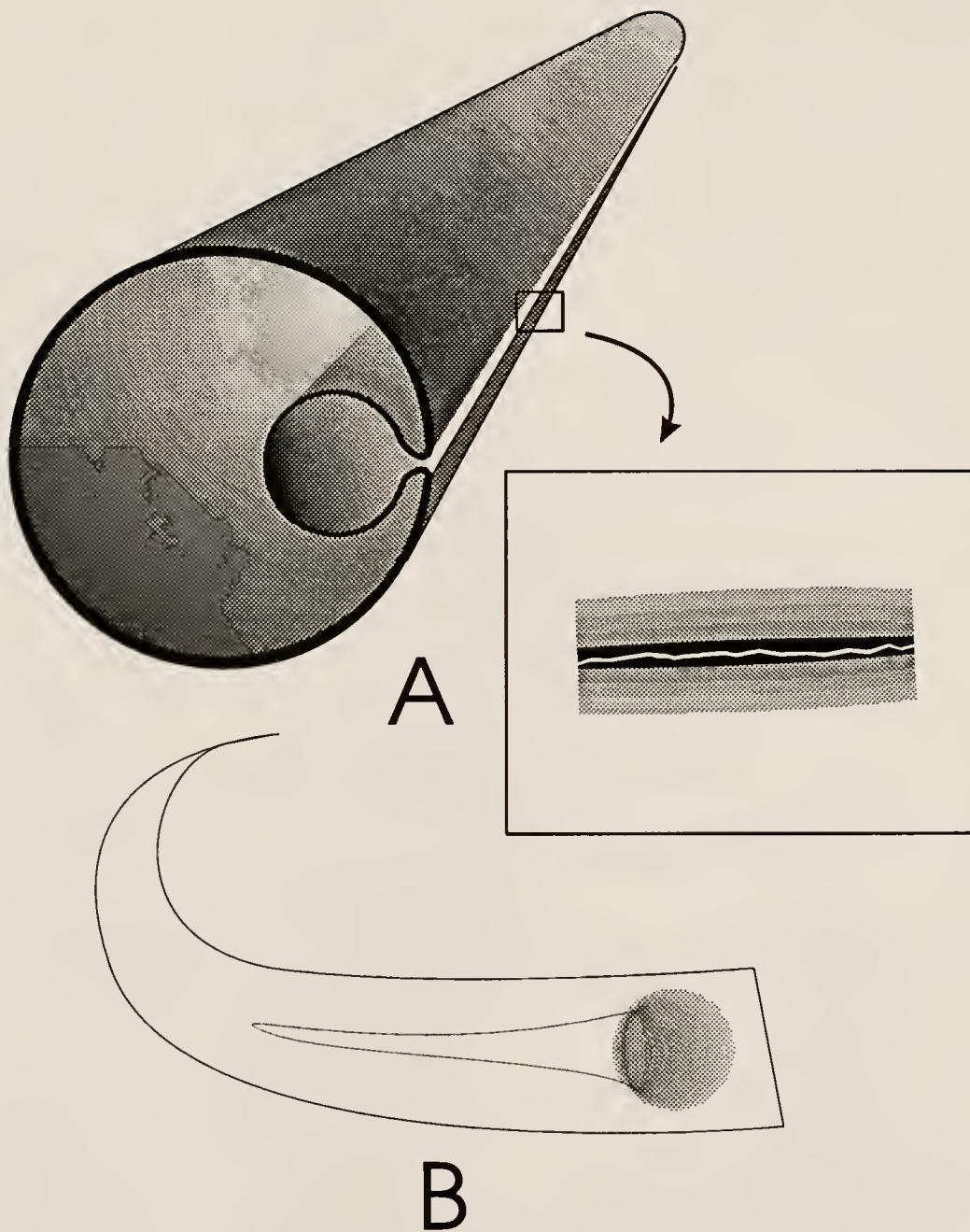
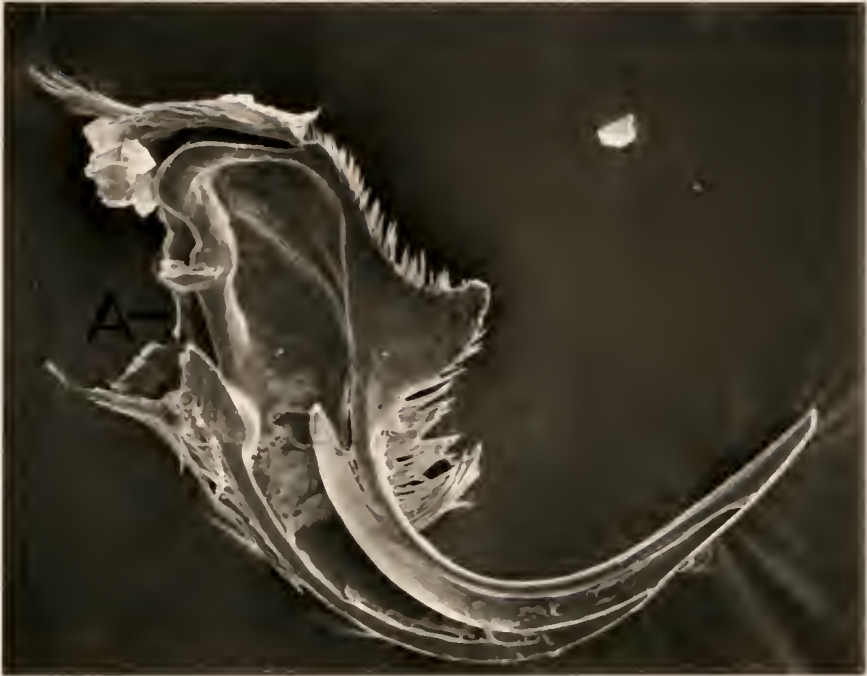


Figure 12. Mandible of Pyractomena larva longitudinally shaved. A) Basolateral opening, corresponding to Figure 9B.

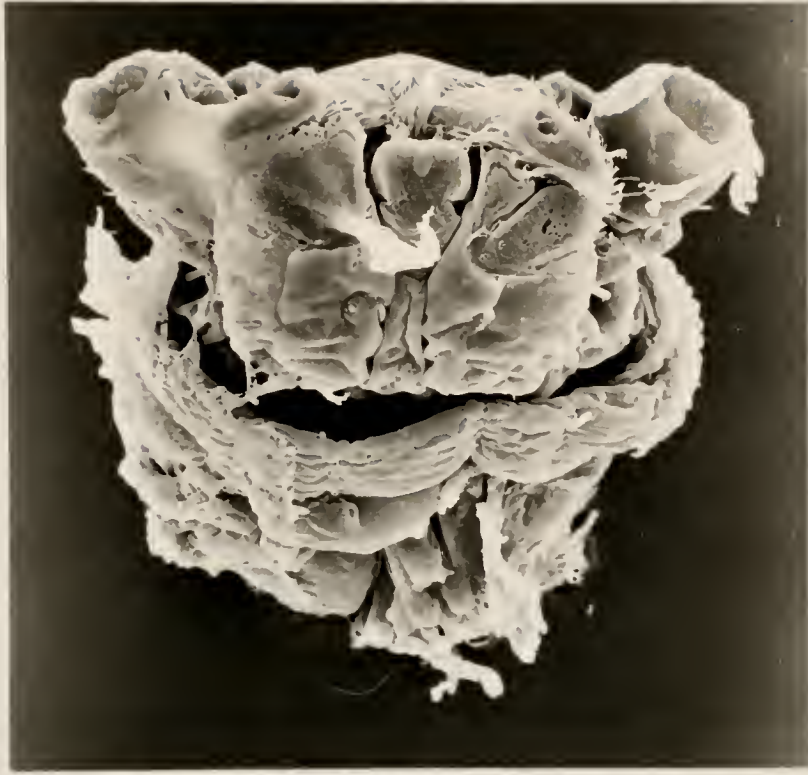
Figure 13. Mandible of Pyractomena transversely shaved. Ventral view.



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Figure 14. Whole mount of a 1st instar P. collustrans, freed from the embedment plastic after being shaved to the basal half of its mandible.

Figure 15. Close-up of mandible in Figure 14.



CHAPTER 3 METHODS

Overview

Specimen processing, from collection to photograph in hand, is a long, laborious task, likened by Hayat (1986) to the bottom of an inverted pyramid. Eggs were deshelled in batches and the embryos were fixed, rinsed, dehydrated, and mounted for SEM or embedded for TEM/LM. In some cases extensive serial sectioning and staining was required of several specimens in order to compare the developmental status of the whole mandibular structure for each. In the youngest embryos, for instance, mouthpart anlagen are so small and amorphous that in order to identify which contour curvature of any given photograph belongs to which mouthpart, a three-dimensional reconstruction needed to be assembled from photographs taken of all cross-sections (Figure 16). Larvae were studied for external indications of the moment of apolysis so that the youngest pharate larvae ("apolytics") could be secured.

The number of specimens used for SEM and TEM was about 150, and the number of useful cross-sections generated reached about 200, a number so large as to require that, whether for

3-D reconstruction or not, at least half be photographed at different magnifications for later collation.

The need for photography decreased as it was gradually determined what is significant and what is not. Negatives and positives were developed by hand.

Collection

Availability of collection and ease of rearing dictated which species could be used. Tenebrio molitor (Tenebrionidae) was selected to fulfill the convention that the same assay be required of an outgroup for comparison.

Lycidae

Calopteron terminale (Archer and San Felasco Hammock, Alachua Co., Florida, January) provided pupae and adults. Their larvae feed underground and are rarely found in numbers. Pre-pupal larvae come to the surface and congregate on the underside of a rock or fallen branch to overwinter as pupae and were collected in this dormant state (McCabe and Johnson 1980; Miller 1988; Young and Fischer 1972). Pupae were housed in a screen cage with a thin layer of moist sand and a rock at the bottom while awaiting eclosion. Eggs were transferred from under the rock to moist filter paper in plastic petri dishes fitted with a screen ventilation window (Figure 17A). After the females died, the sand was washed into a vial rack

(Figure 17C) half-filled with water to winnow for any stray eggs.

Larvae of Lycus lecontei Green (Arthropod Discovery Center, Tucson, Arizona) are available in large numbers throughout the year under fallen saguaro cactus carcasses. Brought to the laboratory, they were provided with "cookies" of dried saguaro flesh (chunks whittled down into discs). Apolytics were identified by their color. Freshly tanned individuals have a high contrast to their light-brown to dark-brown mottling, but as a molt approaches, they take on a dull gray, greasy cast. The latter readily commence larval and pupal molting processes when sprayed with water, and become sluggish and unresponsive to pin-pricking. These individuals can be decapitated and verified for separation. Apolysis is long over by the time they cement their anal proleg down. Pupae likewise readily molt to adults and eggs can then be secured from them in the same way as with C. terminale. No external indication of separation, such as retraction of ocular pigment, could be found.

Adults, larvae and exuviae of Lycus sanguineus (Pena Blanca, Santa Cruz Co., Arizona; Davis Mts. Resort, Jeff Davis Co., Texas, July-August) were collected in small numbers. Adult females readily laid eggs. Larvae were found under rocks. Attempts to induce larvae to molt failed.

Three unknown lycid larvae were collected under dead pine bark in Gainesville in February. Two were reared to Plateros

sp., and the third was preserved in alcohol for low magnification study.

Archbold Biological Research Station (Lake Placid, Highland Co., Florida, May) was scouted for Lycus lateralis (Melsh.), but the population could not be located. It was either inactive, or extinct in the area at the time.

Lampyridae

Adults and larvae of Photuris A (Lloyd 1965) (Gainesville, July) were collected by their lights at night. Housed as above, they provided eggs and one pharate pupa. It seemed interesting to process this specimen also, for a glimpse of the change from larval mouthparts to adult mouthparts.

Housed as above, males and females of Photinus collustrans (Gainesville, March) produced eggs. Larvae are too difficult to find in the wild (Wing 1988), and attempts to rear them from egg to second instar failed.

Exuviae of Pyractomena lucifera and P. borealis (Gainesville, January) were collected off tree trunks (Buschmann 1984).

Tenebrionidae

Tenebrio molitor was auditioned as a non-cantharoid subject because of the ease with which it can be cultured. Stock was obtained from Rainbow Mealworms Inc. (Compton, California) and reared in plastic shoeboxes with 2 inches of

flour, bran and yeast as a nutritious support matrix. Potato slices were added thrice weekly for food and moisture. Larval and pharate larval mandibles were examined for any indications of composite structure.

Various approaches were tried to develop a method for determining whether any external indications of apolysis exist. As a first attempt, these slices were turned over and the writhing masses of larvae were scanned for individuals that might show indications of onset of molting. Sufficient time was spent to reach the realization that such an approach is futile.

As an alternative, groups of 3 mid-instar larvae were isolated in media vials, the bottoms of which were tightly fitted with a wafer of fresh potato. The vials were held in stacking trays, capacity 30 (6 rows x 5 rows). Two such trays were prepared. With this arrangement, larvae on top of their wafer floor scraping for food, a total of 180 could be scanned rapidly for any indications of onset. After several hours, the earliest post-apolytic stage that could be obtained with this approach was a point half-way through eclosion. This indicated that the apolysis-ecdysis transition is rapid and indiscrete. Groups were increased to 4 and 5 per vial, but results did not improve. Other difficulties with this approach were the drying out of wafers and the accumulation of excrement in the plane of feeding.

A final approach, barely satisfactory for the needs of this study, was to place several dozen larvae in a 3" petri dish fitted with two $\frac{1}{8}$ " thick wafers that together covered about 80% of its floor. By turning them over in alternation, one was eventually spotted that was slightly curled and lying on its side. Removed to the microscope stage, the stemma, spiracles and other key areas were monitored for indications of separation.

This larva set up anteriorly-directed, undulative spasmodic movements within 3 minutes of the above cessation of feeding, and the outer cuticle split mere seconds later. No anatomical indications of separation were detected. It was decapitated immediately and processed for microscopy.

A second larva was obtained in the same way for additional microscopy technique. Because of the rapidity of the molting process, it is suggested that some electronic means of detecting onset, such as tapping into the nervous system, might be the best way to study the pharate larval stage of this species.

Processing

The summary of microscopy technique below is designed to convey the special considerations that arose in this study and assumes the reader has an understanding of the basic regimen.

Fixation

In my hands, a modified Karnovsky's consisting of 2% gluteraldehyde, 1.5% formaldehyde, 1.5% sucrose and a few drops of acrolein in 0.1M sodium cacodylate buffer yielded satisfactory results for embryos and teneralis. Specimens were left in cold (4°C) fixative overnight, then rinsed with buffer for 1-2 hours. Carnoy's, Bouin's and FAA (formal acetic acid) were not used because they are coagulant fixatives that create serious artifacts. Although still widely used today, they are strongly discouraged by leading histologists (e.g., Hayat 1981, 1986).

Specimens are easiest to divide up for SEM or TEM/LM during the buffer rinse. Those selected for SEM can then be osmicated as it does enhance electron opacity. Osmication is not recommended for TEM and LM because the resulting light opacity makes it too difficult to orient the specimen relative to the block face (Figure 18). Also, osmium reacts unfavorably with LM dyes.

Specimens usually float in fixative because of cuticular waterproofing and air in the tracheae, and attempts were made to make them sink. The surfactants known as Aerosol O.T.* (Sargent) and Photoflo* (Kodak), vacuum, and gentle, downward prodding with a rod were tried with variable success.

Embryos

Staging. Insects such as Drosophila have features that allowed investigators to divide the entire embryonic process up into numerous periods according to the appearance of conspicuous anatomical landmarks (Campos-Ortega 1985). They are available in large numbers, have a low yolk content and will complete maturation while submerged in refraction-enhancing oils (paraffin oil, Voltalef oil, immersion oil). I examined and tested my animals for the feasibility of staging as a preliminary to the main thesis, but they possess none of these features.

However, incipient segmentation is visible in anatreptic specimens without dechoriation, and the mouthparts are mere bulges at this time (Figure 19). Therefore, only stages following this point were required and, given enough specimens, a sufficient spectrum of intermediates could be harvested by processing a large number of batches indiscriminately. Age differences were then inferred by the length and curvature of the mandibles.

Dechoriation. Dechoriation was needed only initially to learn key features that allowed for a rough estimation of age. Thereafter, transmitted light sufficed. It was advantageous to leave some rings of chorion intact for strength, oxygen and waterproofing. Fully or partially dechorionated eggs desiccate quickly and require that a moist atmosphere be maintained. Using upward motions, the egg is

rolled on double stick tape within a corral that will catch the egg should it flick off the adhesive (Figure 17B). After the desired amount of chorion has been removed, the egg is rolled off the tape and lifted with a camel's hair brush.

Cracking the eggshell. The egg is compressed with tweezers that also embrace a pin ca. 0.3mm in diameter (Figure 20). This protects the embryo from being squashed. Cracking is best done under buffer or fixative. Dechorionated eggs are flaccid and cannot be cracked in this manner. Also, they do not fix with the phase-partition techniques of Zalokar (1971, 1977) either, probably because the vitelline membrane is impervious to heptane. This being the case, the only method available for accessing the internal milieu of a dechorionated egg is to poke holes with a pin and try to rip the shell without damaging the embryo within.

Rinsing. Agitating the opened egg in buffer will rinse it of yolk that otherwise adheres to the embryo during the fixation process. This is especially desirable for SEM preparations.

Fixing. Opened eggs were soaked in fixative for at least an hour before deshelling. This gave the embryo additional strength to withstand any distortion that may occur while peeling. Once freed from the eggshell, the embryo was left in cold fixative overnight. Only a few specimens were kept in each test tube so that those on the bottom were not fixed in a compressed state by specimens on top of them.

Deshelling. Deshelling is required for all microscopy techniques, and should be done under fixative. The most difficult aspect is freeing the shell from the mesothoracic spiracles which adhere tightly to the air pores. Careful ripping and shredding of the shell with one pair of tweezers while holding with another is the usual method.

Fragmentizing the embryos. It seemed advisable to cut or lacerate the embryo to allow rapid entry of fixative. Controls were not run to determine its actual benefits, but when the mouthparts are hidden in the curl of the body, then the body must be broken in half anyway. Therefore, a decision must be made as to whether to fragmentize before, during or after fixation. The uninsulted body probably stands up best to the harshness of the dehydration process. Further, whole embryos are easier to position on a stub, and their brittle nature allows for clean fracturing of unwanted body parts (Figure 21). Some of the SEM preparations showed blebbing, perhaps due to gasses entering between folds of cuticle during critical point drying (Figure 22). For embedment preparation, it is sometimes useful to remove unwanted body parts so that the cross-sections are less confusing, and so that the knife need not endure the cutting of other body parts before it reaches the mandibles.

Larvae

Tweezers were sanded to ultra-sharpness for the delicate task of teasing the apolysed head out of its exuviae. The major obstruction was pulling the crop through the narrower pharynx (Figure 23). Their mouthparts and those of teneral were cut off at the frontal region and taken directly into fixation.

Fixation was bypassed for mandibles of alcoholed larvae and exuviae; these were taken directly into dehydration, which helps to clean their surfaces and avoids taking water into the EM column. The hard exoskeleton of these and live, post-teneral larvae make TEM prohibitive. Fixative cannot penetrate into the mandible interior, and knives, whether glass or diamond, cannot stand up to it.

Dehydration

A full ethanol series (25-50-75-95-100%) was required after rinsing of fixative. Specimens were left in 95% overnight. Critical point drying was used instead of hexamethyldisializane (HMDS, Nation 1983), although it can give satisfactory preservation also (Figure 24). HMDS is convenient when dehydrating hard mandibles, where, as mentioned above, the concern is to avoid bringing water into the EM column. Whole embryos can be bagged with fine mesh

netting for critical point drying. Fragments can be held in a Beem[®] capsule modified to hold Millipore[®] filters (Figure 17D).

Mounting for SEM

The specimen holder used in dehydration was placed under the microscope in the same focal plane as the gummed SEM stub so that transfer could be done quickly and smoothly. Dried specimens were arranged so that the area to be viewed faced upward. Lifted with one tweezer tine made tacky by scraping on skin of fingers, they were then touched down on the stub in even orientation. After sputter coating, each row was underscored with a razor blade. This organization minimized goniometer movements and greatly facilitated the systematic perusal of each specimen (Figure 25).

Embedment for TEM

Quetol or hard-formulation Spurr's is recommended for so hard a structure as a mandible. A plastic-acetone gradation of 25%-75%-100% was carried out at room temperature. Infiltration was protracted to several days for each step since whether tanned or not, the narrow, cuticularized passages are very difficult to infiltrate.

It is usual that the specimen sinks to the bottom of the mold during polymerization. This complicates trimming for two reasons. Firstly, the bottom is a coarse surface, leaving an opaque, frosted imprint on the hardened block. The frost, and

the meniscus at the top, do not permit viewing to estimate the orientation of the head. Secondly, trimming requires that several millimeters of plastic surround the specimen, so that there is room for a block face to be constructed around the desired area of approach, and for a base where glue is applied. Blocks can be turned upside down in their molds and a fresh, thin layer of plastic added. This layer will be flat, transparent, and an interface will not develop which might otherwise complicate sectioning.

Mounting on a microtome chuck stub

Because of the expected complexity of mandible embryogenesis, it seemed likely that accurate knowledge of the angle of approach in the microtome would greatly facilitate micrograph interpretation. The best references for this are the ocular pigment masses, which can be eclipsed through any of the five sides of the block apex. Eclipsing through the block face is probably best, yielding a lateral approach to long sections of the outer mandible (Figure 18). The inner mandible is then reserved in case remounting at a different orientation is desired. LKB 8800 Ultratome microtomes, used in this study, are designed so that the chuck can receive a cylindrical stub, to which the block is glued. Since it is best that the block face be perpendicular to the axis of the chuck stub and well supported at its base, the base needs to be perpendicular also. Because of refraction, varying

translucency of body parts, prismatic reflections and other difficulties with viewing specimens en bloc, this requirement is very difficult to satisfy.

To accomplish it, the block face plane is estimated and marked with a razor blade under the dissecting microscope. A coping saw is used to trim very conservatively using this guideline. Specimen-to-base distance should be about 10mm, to face, 5mm. The block is then mounted in a flat-jawed chuck and polished with the microtome. The meniscus is removed at this time. Trimming continues on the microtome with occasional recourse to another dissecting microscope until the specimen is close enough to the face that it can be viewed with minimal refraction, yet deep enough that the face angle can be corrected when, inevitably, the compound microscope shows that it is incorrect. To view under a compound microscope sometimes requires whittling the stub (Figure 26). Now that the eclipse is virtually true, the block can be glued to the stub. The stub is stood on a slide and the block is propped with fragments of plastic such that the eclipse can be viewed through a compound microscope. Cyanoacrylate (Crazy Glue[®]) penetrates between the fragments with a low capillary tension and will not disturb the prop. Now that the inter-ocular axis is parallel to the stub axis, the stub can be mounted in the cylindrical chuck and the face can be honed so that it is perpendicular to the microtome arm advance. The sides can now be trimmed to a rectangle so that the specimen can be viewed

through them under the compound microscope without prismatic reflections. Older compound microscopes generally have enough clearance to give at least 400X to the apex of a stub lying on its side. The final trimming strokes to a trapezoid shape can be made after the shape of the mandibles is noted or, preferably, photographed. Tedious recourse to and from microscope and microtome can yield a ca. 97% accuracy in angle of approach. Trimmed blocks are very fragile and should be housed in a cropped Eppendorf* tube (Figure 17E).

Sectioning

The late embryonic P. collustrans mandible is about 40 μ in basal diameter allowing for a maximum of 800 500Å sections, far beyond what is required for this study. It was determined during development of methods that the best sectioning pattern is semithin (1 μ) for LM and ultrathin (silver, 500Å) for TEM in alternation (Figure 27). Semithin sections of cuticle-coated specimens can be fragile enough that at least two should be taken at a time so that at least one might reach the microscope intact. If more than two are taken, the risk is run that a critical area of the specimen will be passed over without securing an ultrathin section for it. Plastic adheres to the cuticle very poorly and parts of semi-thin sections can fold over or fall out if the section is fished from the boat with a camel's hair. Use of a platinum wire loop solves this problem. Sections are floated onto a drop of water on a slide,

dried, stained with Toluidine Blue in 1% sodium borate buffer, rinsed, dried, and mounted with Pro-texx[®].

The block face is best prepared for the silver sectioning to follow by advancing short of the second full micron so that a third pass gives ca. 0.1μ . The only consideration involved in deciding on the number of ultrathin sections to take at a time is abrasion to the cutting edge. At least two are necessary since the one adhering to the cutting edge is liable to be damaged when teased. 1x2 slot grids are required for TEM since there is only one locus of interest per section. Uranyl acetate-lead citrate are used as post-stain. In most cases all need not be post-stained. LM slide libraries, as well as SEM photographs, serve as the primary focus of research in being well suited for the gross orientation required of this study. TEM grids can be resorted to for a more detailed look where necessary.

Viewing

Exterior and interior views of post-embryonic mandibles can be obtained in a variety of ways. Straight-forward viewing of mandibles under a dissecting microscope requires at least 80X. A Zeiss with 80X, another with 96X, and Leitz with 110X were used in this study. Reflected light can be used with a compound microscope to go beyond these magnifications at the expense of depth of field. This is helpful for cursory examination as it bypasses the processing required by the SEM.

Transmitted light is useful for preliminary examination of lycid mandibles and this view is accentuated with any of the refraction-enhancing oils.

Interior views of post-teneral mandibles can be had by embedding in resin, shaving to the desired cross-section, dissolving away the resin, then mounting for SEM (Figure 12 through Figure 15). The critical point dried embryonic mandible is brittle enough to allow chipping without embedment- the SEM stub to which the embryo is affixed can be clamped in an ultramicrotome even though it is designed for resin blocks. The magnification of the dissecting microscope fitted on the ultramicrotome is too low to observe progress but the edge of the knife can be watched for fragments (Figure 21). It is advisable that photos be taken of specimens before and after this fracturing.

Records

An efficient method of cross-referencing notes and micrographs to specimens was devised in consideration of the fact that specimens are too small to be remembered by some idiosyncrasy, as is done with, for example, pinned insects.

Scanning electron microscopy. A plastic petri dish served as a desiccator jar platform for SEM stubs. It was poked with holes and the holes numbered in reference to a logbook. Specimens were given a number according to their rank and file

on the stub which was entered on a finder sheet (Figure 29) and dialed into the micrograph.

Transmission electron microscopy. Numbers were written on the bottom of block stubs for reference to photographs and drawings that indicate angle of approach and progress. Grid box number was referenced to micrograph number. LM slides were referenced according to the box number of the two grids they are between.

Film

The cost of Polaroid 55 sheet film is currently more than \$2.00 per sheet, while Tri-X Pan 35mm film can be purchased in 100 foot rolls at about 11¢ per shot. The difference in quality is negligible.

Artifacts and complications

Several annoying complications appear in one step or another of the extensive processing required of specimens. These are mostly tolerated as they do not detract sufficiently from the quality needed for this study.

Shrinkage and collapse of tissue occurs quite often but usually in areas other than the mouthparts. Distortion of this sort is easy to recognize and does not cause misinterpretation.

So-called "post-stain garbage", probably $\text{Pb}(\text{CO}_3)_2$ (Figure 73), appears as opaque circles that are easily

recognized from actual specimen structure. It is best avoided by testing the lead citrate preparation on a blank grid before using it on those with specimens. Extensive rinsing should also be employed.

Knife marks result from the use of glass knives for tough material such as cuticle. Even diamond knives would not stand up to this material for long, and are far too expensive to be subjected to such abuse anyway. Knife marks are a cosmetic problem, and to avoid them would increase the consumption of knives by perhaps tenfold.

Shrinkage of semithin sections is common and causes annoying transverse ripples (Figure 57, Figure 63). In most cases these too are only cosmetic and for the level of interpretation required in this thesis, can be ignored.

Scanning electron microscope stub mounted embryos can be cleaned of adhering yolk and other debris (Figure 30, Figure 31, Figure 32) by dabbing with a tiny blob of adhesive held in the tweezer tines. This is a delicate operation, but may be necessary if an important area is obscured. Specimens to be cleaned should be carefully lifted to another stub so that others are not sputter-coated twice. Sonication might also work during the buffer rinse, this was not attempted.

Microscopy is now a highly sophisticated technique. However, the results they produce have interpretative limits. For cytodynamics, cytochemistry and functional morphology, other techniques must one day be employed.

Figure 16. Cross-section of the head of an anatreptic P. collustrans embryo showing the difficulty in identifying which lobe corresponds to which anlage without 3-D reconstruction.

Figure 17. Materials used. A) Petri dish with window. B) Dechoriation of egg with two-sided tape. C) Vial rack. D) Eppendorf® vial with tip cut off. E) Beem® capsule modified for minute specimens.

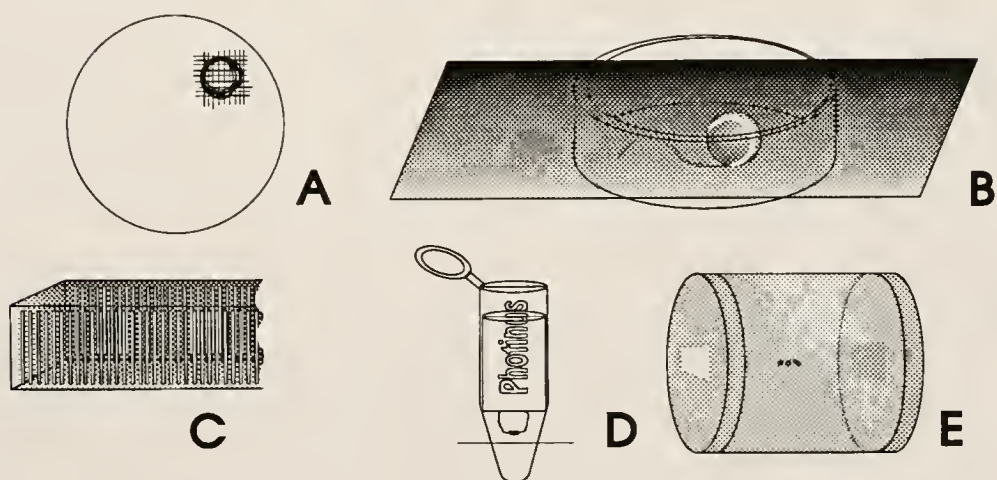
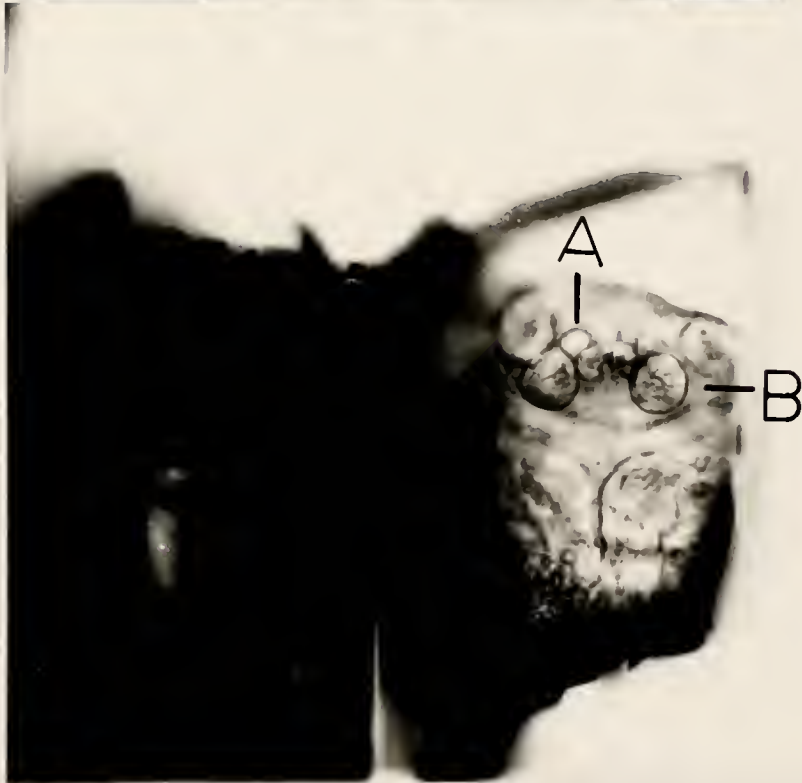


Figure 18. P. collustrans embryo embedded and photographed through a compound microscope. See Figure 26. The ideal orientation is shown, with the eyes eclipsed to the viewer.

Figure 19. Anatreptic P. collustrans embryo, embedded and photographed through a compound microscope. See Figure 26. A) Mandible anlage. B) Maxillary palp anlage.



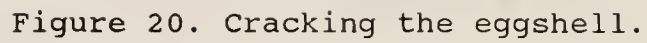


Figure 20. Cracking the eggshell.

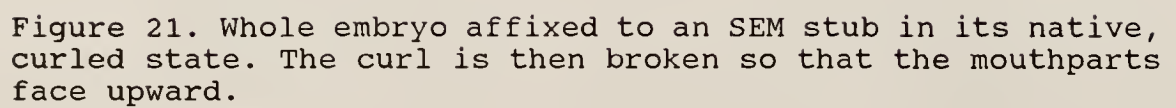


Figure 21. Whole embryo affixed to an SEM stub in its native, curled state. The curl is then broken so that the mouthparts face upward.

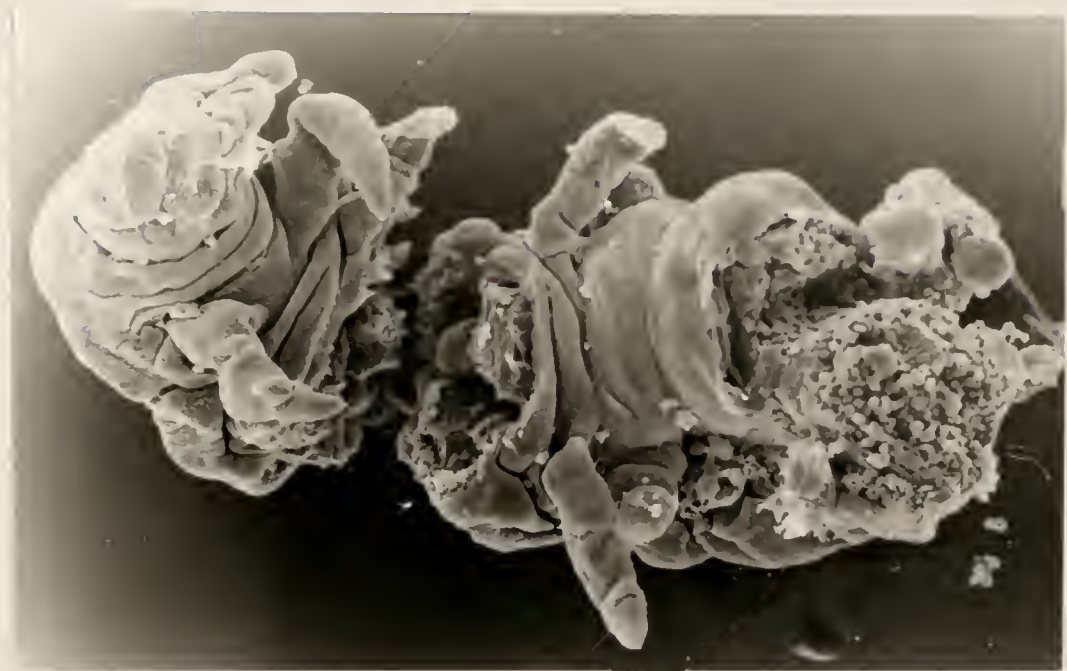
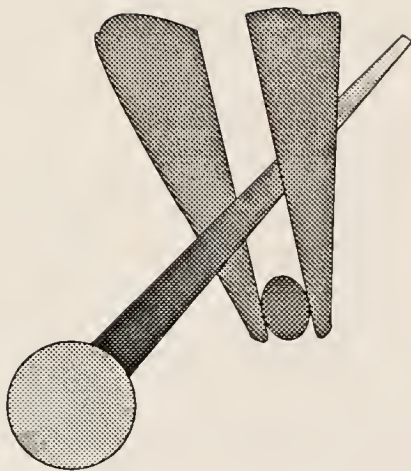


Figure 22. Blebbing of cuticle in an improperly processed specimen.

Figure 23. Pharate larval Lycus lecontei, dorsal view, fixed, then removed from its exuviae. Central gap is where stomodeum passes through mouth when the head is pulled out of the exuviae. A) Labromandibular rudiment. B) Gala. C) Maxillary palp.

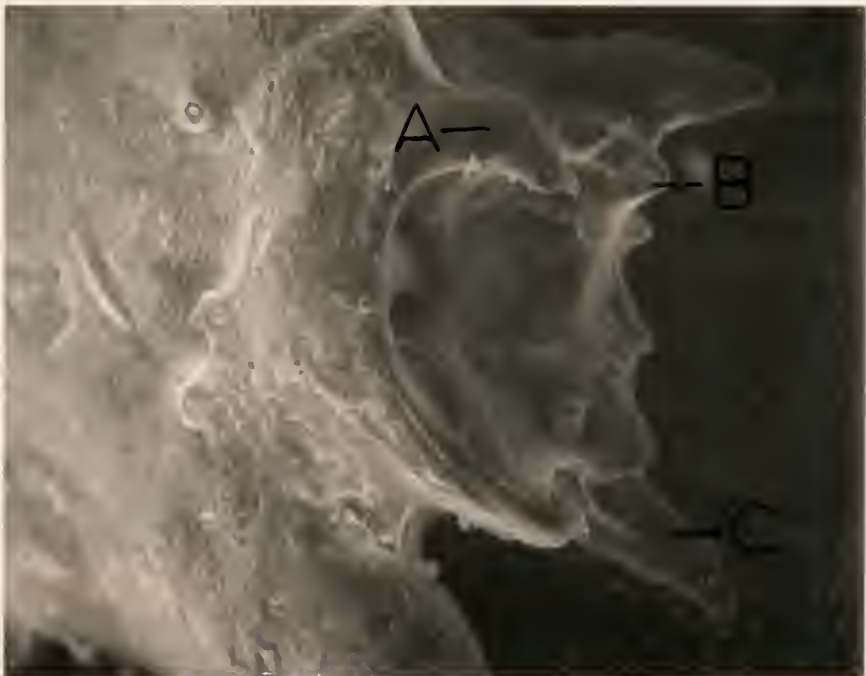
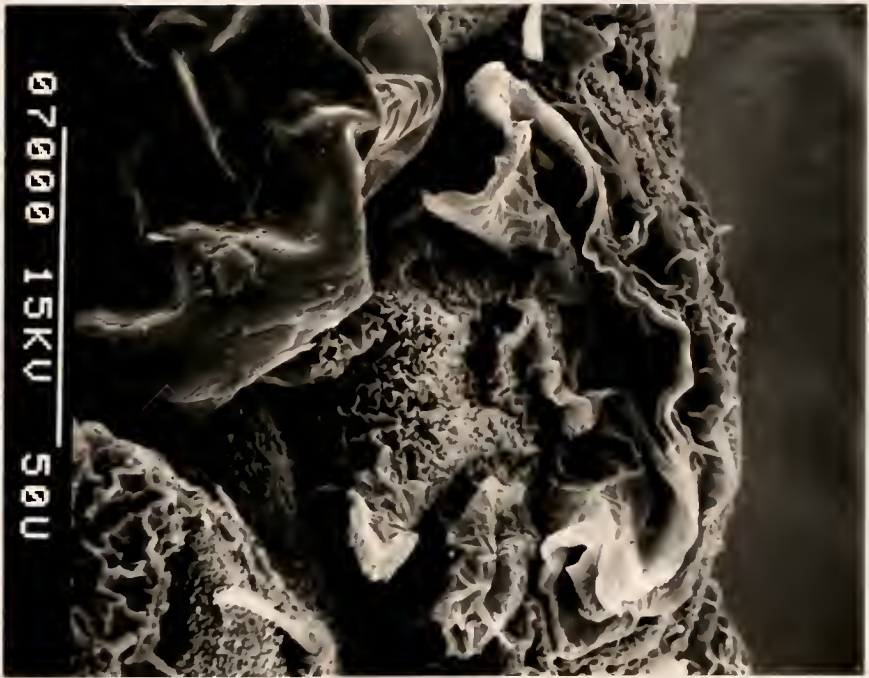


Figure 24. Trial specimen, a mole cricket embryo, dehydrated with HMDS.



Figure 25. Well organized SEM stub with all embryos facing the same approximate way along scored lines so that magnification and goniometer adjustment is not necessary for perusal from one specimen to the next.

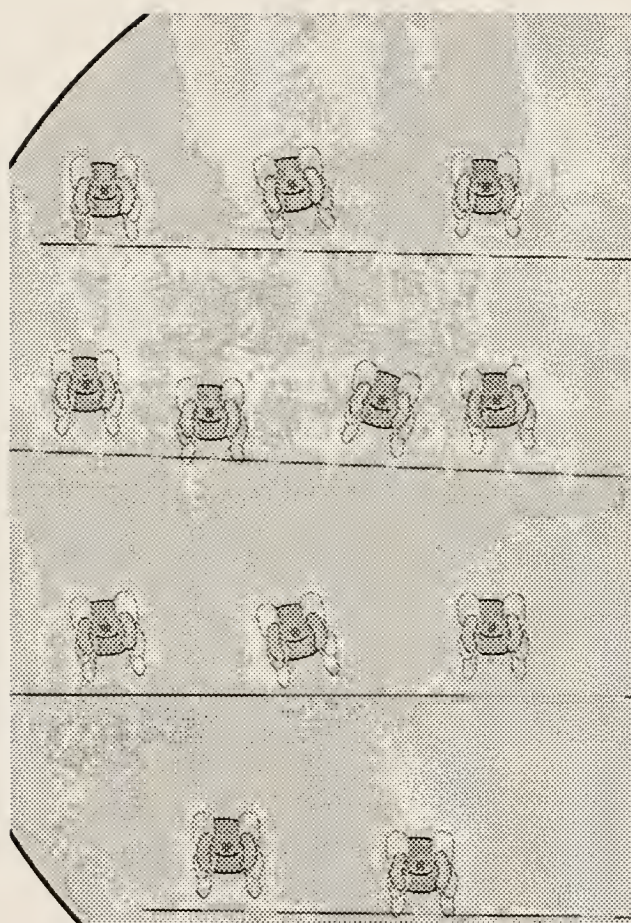


Figure 26. Viewing embedded embryos through a compound microscope. Whittling of the stub was necessary. Figure 18 and Figure 19 were photographed by this means.

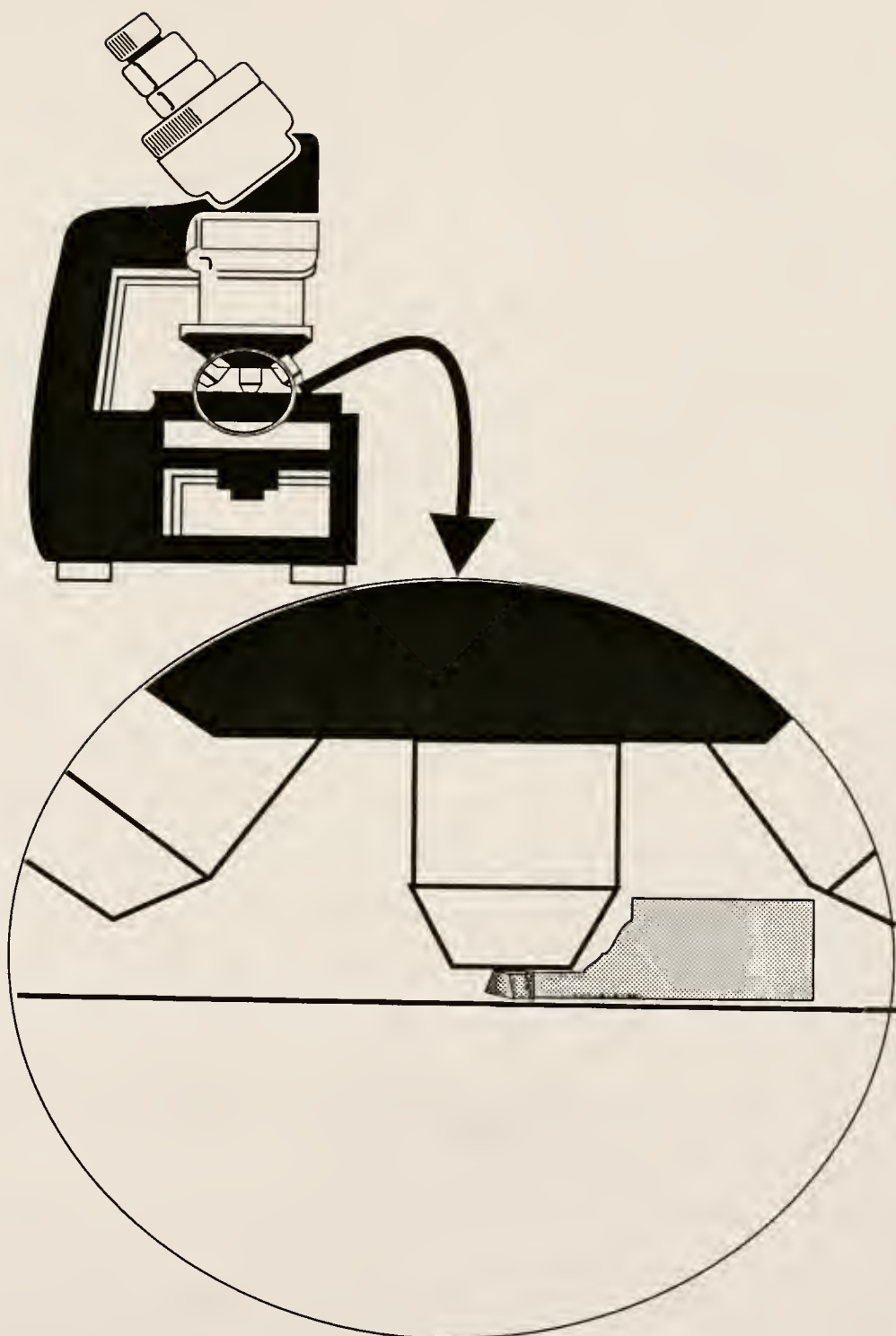


Figure 27. Sectioning sequence, ultrathin alternating with semithin.

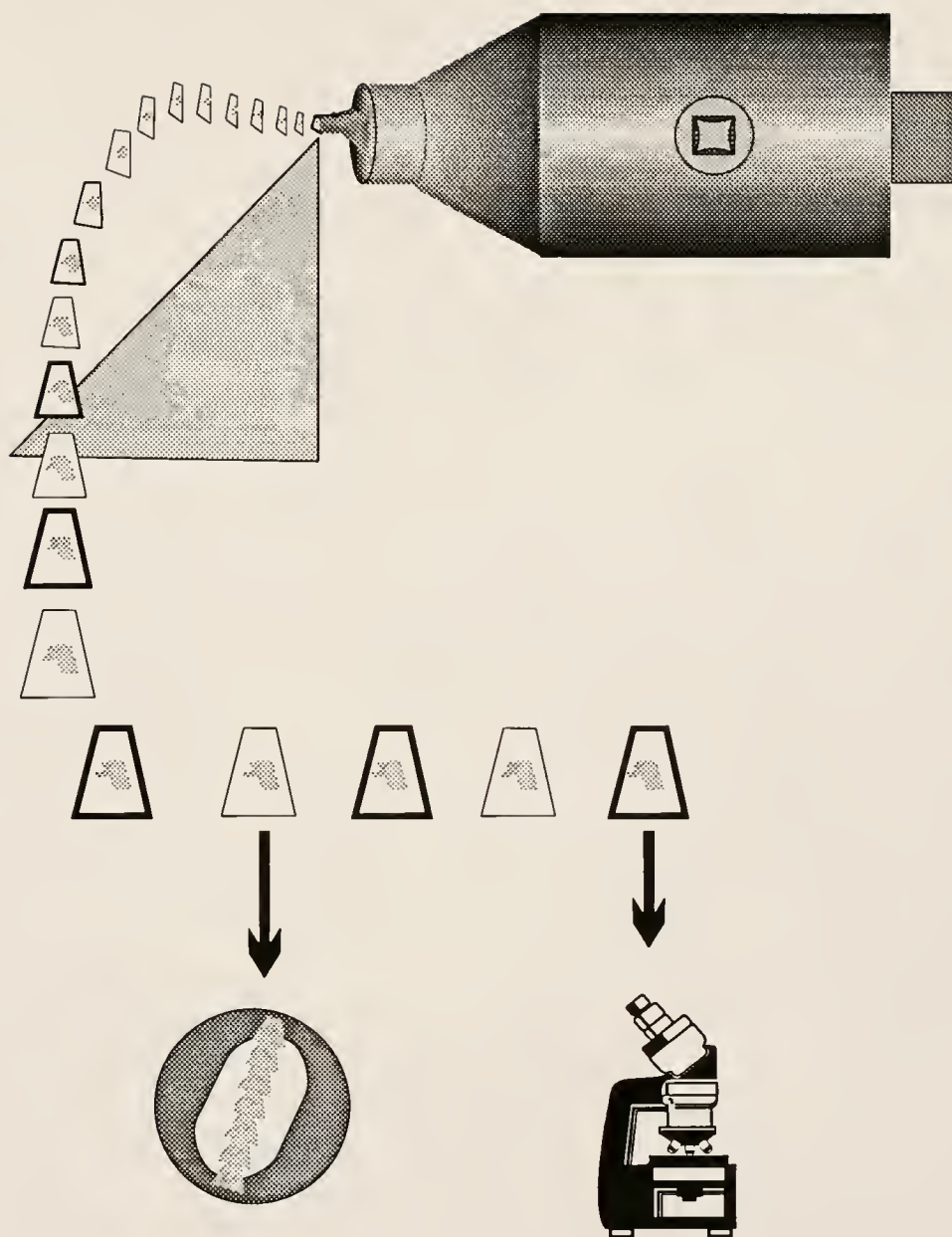


Figure 28. SEM stub mounted in a microtome chuck so that tip of specimen's mandibles can be shaved. Large arrow points to fragments of the mandible tip.

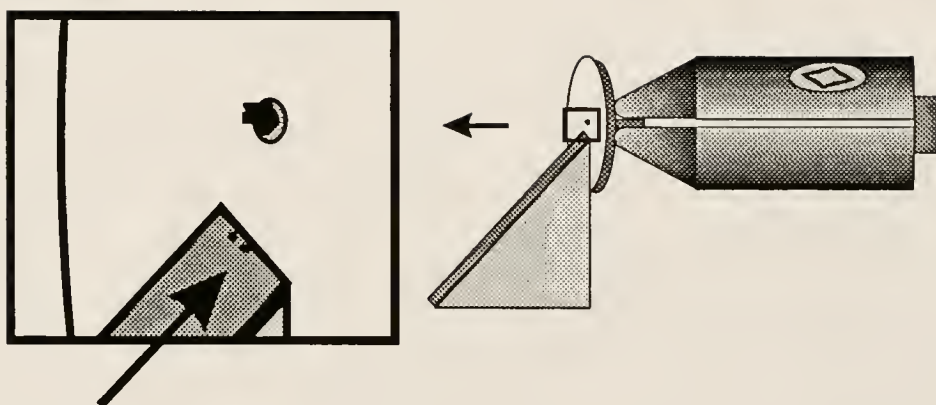
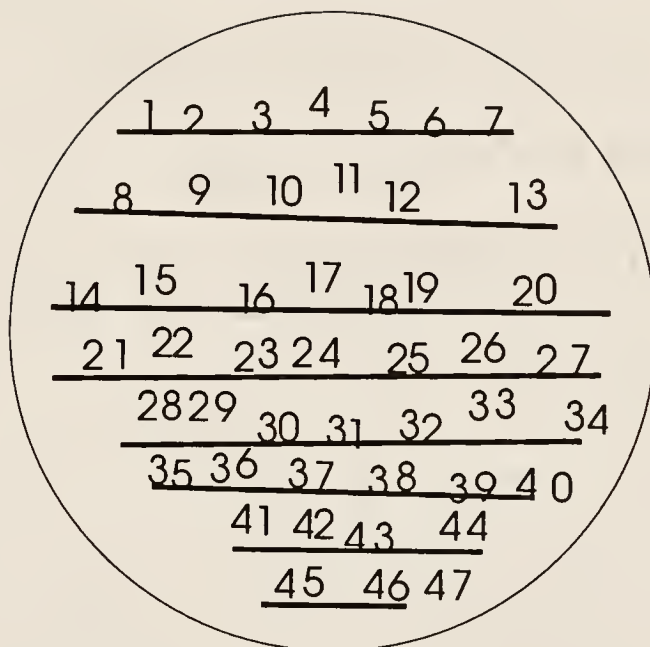


Figure 29. Finder sheet for SEM stub specimens.



1	1 1	2 1	3 1
2	1 2	2 2	3 2
3	1 3	2 3	3 3
4	1 4	2 4	3 4
5	1 5	2 5	3 5
6	1 6	2 6	3 6
7	1 7	2 7	3 7
8	1 8	2 8	3 8
9	1 9	2 9	3 9
10	2 0	3 0	4 0

Figure 30. Blob of glue held between tweezer tines to clean the stub mounted embryo.

Figure 31. P. collustrans embryo obscured by adherent yolk and other material.



Figure 32. P. collustrans embryo cleaned by the method in Figure 30. Such results are rarely so good as most debris is firmly dried to the embryo. A) Invagination.



CHAPTER 4

RESULTS

Normal lampyrid and lycid embryos undergo the typical blastokinetic movements, anatrepsis and katatrepsis. Anatrepsis occurs when the body elongates to the extent that the abdomen curves over the dorsum of the body, as in Figure 33A. Katatrepsis follows, and is characterized by a convulsion that reverses this curve so that the abdomen is positioned ventrally, as in Figure 33B. During this convulsion, the embryo breaches the serosa (Anderson 1973). Throughout this dissertation, I shall refer to embryos that had not undergone katatrepsis prior to fixation as "anatreptic" embryos. Similarly, "katatreptic" embryos are those that have undergone katatrepsis.

Two blastokinetic mutants were discovered among the lot of Calopteron eggs harvested, one in which katatrepsis is omitted and another in which katatrepsis is accomplished but the embryo fails to breach the serosa and develops within it (Figure 33).

Mature Photuris eggs possess extreme tolerance to inanition. Several of a batch were mistakenly allowed to dry. They suffered as much as 80% loss in spherical volume when the shell collapsed (Figure 34). The embryos could be seen in

severely contorted postures by the coloration of the eyes, setae and mouthparts that had been achieved. Amazingly enough, when rehydrated, the embryos slowly expanded and eclosed to healthy, ambulating, luminescing 1st instars.

It will be easiest to begin this exposition of mouthpart ontogenesis with a description of the finished products, then follow with the earlier stages leading up to it. The term "syntrophium" will herein replace the structure classically called "mandible."

The Larval Lampyrid Syntrophium

On encountering a prey item, such as a snail, lampyrid larvae will attack by impaling the foot with a persistent bite that causes the foot tissue to lacerate when the snail withdraws (Cicero, unpublished observations). Imbibition of the slime is presumably through the canal, but no experiments have been undertaken to prove this. Their syntrophia articulate laterally only; they cannot perform dorsoventral grinding movements.

The mature syntrophium

This structure is very long, ~4X the basal diameter of the antenna. It is a symmetrically paired structure with a dorsally deflexed (Figure 35A), interolaterally expanded base (Figure 38B), a ventrolateral acetabulum/condyle articulation, a shank that curves inward 90° and upward ~10° to an acuminate

tip, a minute retinaculum in the center of this curvature, and an internal canal. The tips angle further upward because the whole affair is slightly rotated about its long axis (Figure 47).

Transverse and longitudinal series can be compared for most of the particulars required to understand its internal structure (Figure 35 through Figure 46). The canal is married to the inner wall of the cone for its entire length. It opens to the buccal cavity dorsally, extends through the cone's shank, and ends in a slit-like pore. The pore is located at an outer, anteapical site (Figure 46, Figure 47, Figure 48). A seam can be detected near the pore in mid-instar Pyractomena and Photuris.

At the base, where it opens to the buccal cavity (Figure 42), the canal does assume the motif of an evagination as modelled earlier (Figure 11A). The tip, distal to the pore, is entirely cuticle, suggesting that cells back off as they lay the cuticle down.

At the cytological level, most striking is the high-nucleus-to-cytoplasm ratio in this area. These cells, continuous with those of the head epidermis, run up into the shank where they undergo extreme attenuation to accommodate the apical diameter of the tip (Figure 49). A large, apparently disc-shaped, amorphous area occurs near the base which, oddly enough, appears to be open to the cytoplasm of the cells surrounding it (Figure 38), as is a similar region

noticed in the antenna. There may be a partition present that is too fine for the processing to detect. This region resembles that found in the aphid stylet, mentioned in Chapter 2 (Forbes 1969:560).

Innervation is indicated through to the pore by dendrites. These probably service the campaniform sensilla and branched seta that occur at the base of the pore (Figure 50 through Figure 52).

The younger syntrophium

For the youngest specimens, extensive cross-sectioning was necessary to determine which lobes were facial and which were cervical. The syntrophium is the lobe just above the gala and below the front (Figure 53 through Figure 56). It is produced by a compound epidermal cell field that grows outward in length as a short, globose structure, rounded at tip and preserving a spacious haemocoelic lumen. Its apex begins to invaginate (Figure 32, Figure 57, Figure 59). The lumen fills with cells as curvature commences so that only narrow conduits are present which, presumably, are for haemolymph transport (Figure 67). An extraordinarily high nucleus:cytoplasm ratio is noted at this age also (Figure 58).

Sections were scrutinized for the mechanism by which curvature is accomplished. Possibilities include a greater density of cuticle along the outer face than the inner face, or perhaps a ligature that ties the tip to the front. No

indications were found. There is also no indication of any association between the invagination and curvature or canal formation. No developmental consequences of this invagination were detected.

A molt occurs at about this time of curvature initiation, and the exuviae of the stomodeum can be seen in front of the mouth (Figure 57, Figure 59). At commencement of curvature, the incipient canal appears at the bottom of the dorsal deflection and progresses in a distal direction through the center of the shaft (Figure 60 through Figure 67). The dorsal deflection is far too subtle to identify at first appearance. Most striking at this time is the generation of highly electron dense material in cell membranes that are in the proximity of the presumptive canal path. The canal is fluid filled and results from a roughly axial zone of cells that present microvilli toward the center and pull away from each other. The lengthening of the canal continues during curvature until, presumably, it breaches to the exterior anteapically and then lays down cuticle that forms the lining of the canal. This last moment in attainment of continuity was not detected in the cross-sections collected.

The Larval Lycid Syntrophium

In contrast to lampyrids, lycid larvae are sedentary feeders, poking the syntrophium into decaying organic

material, slimemolds, fungi and other sources of food depending on the species (Lawrence 1991).

The mature syntrophium

This composite structure is very short, only ca. 1 to 1.5X the diameter of the basal antennomere and retractable between prognathous- opisthognathous positions. When in the posterior position, the shank proceeds ventrally for a short distance, then curves like an elephant tusk directed caudad-gradually diverging outward, then curving downward to an acuminate tip (Figure 68). Figure 69B shows a strong apodeme that marks the ventral limit of the epicranial half, uniting it with the labium (Figure 69, Figure 90).

Four paired structures can now be identified as functional components- the labrum, mandible, gala and the maxillary palpifer. In Plateros the latter two are independent. In the other genera studied, Calopteron and Lycus, the gala is crescent-shaped in cross-section (Figure 7C), and this crescent is upwardly open to receive the mandible and labrum as their sheath. The palpifer assumes a concavity where it rests against the tripartite complex (Figure 7D and E). Both the gala and palpifer are rugose, setose and easy to recognize as separate from the mandible.

The association between labrum and mandible can be followed in cross-sectional series (Figure 70 through

Figure 80). As noted by initial inspection with LM (Figure 8E), the labrum is longitudinally parted into two rugose, setose, spindle shaped sclerites which are separated from the front and the mandibles by a membrane. It emerges from the anterior edge of the head as a transverse fold as does any other appendage, such as a wing. Its ventrolateral surface infiltrates the lumen of the mandibular arc and coextends with it as an internal stylet. When the dorsal and ventral exuvial halves of the head are pulled apart (Figure 8), this stylet breaks off at its base and appears to be a separate structure.

Nested in the stylet is a rectangular, cuticular sheath that contains two neural tubes. These tubes are wrapped in glia and contain a meshwork of microfibrils. The sheath marries and unmarries itself from the stylet as it travels the stylet's length (Figure 73, Figure 75).

The labrum and mandible emerge from the head separately and remain separate for their entire length. That is to say, no cross-bridges were detected in any cross-section, and the two can be teased away from each other suggesting that there is no adherence. The gala, too, remains discrete (Figure 76). All three are cell-filled. In the L. lecontei gala, the cells end before the tip, which then becomes an alveolate matrix created by dehiscence of spheres (Figure 81 through Figure 83).

Figure 86 through Figure 93 show different profiles of a pair of exuvial halves stretched over a pinhead (Figure 84) so that the mouthparts are exerted. These supplement the above cross-sectional series to give a full understanding of the arrangement at this age. The mandible issues from the pharyngeal walls as a transverse process, but the inner margin abruptly rolls over as the structure undergoes a bend to hypognathy. As can be seen in the close-up (Figure 87), the roll is to the interior. Following the roll distally to Figure 85, it is seen that the mandible is twisting so that the roll takes this originally inner margin all the way under itself. The originally outer margin rolls also, although only slightly so as to curl into the flute. Figure 93 is an inner ventral picture showing acceptance of this pair by the gala.

The younger syntrophium

The youngest embryos processed were anatreptic Lycus sanguineus and Calopteron terminale. At this time the labrum is a shallow, bilobed swelling, higher than wide (Figure 88). A minute flap, presumed to be the hypopharynx by comparison to older embryos, appears between the mandibular lobes (Figure 89).

Scanning electron microscope processing of older C. terminale embryos yielded

clear views of mouthpart composition (Figure 89 through Figure 93). As with the post-embryonic mouthpart arrangement,

both the dorsal and ventral surfaces of the labrum are layered with cuticle; the labrum actually emerges as a transverse fold. Its lateral margins autonomously take on an inward fluting and grow out on top of the mandible. The tip of this flute coextends with the tip of the mandible.

To be certain of the association of these two minute characters, the SEM stub was mounted on a microtome and the tips were chipped (as in Figure 28). Figure 91 shows 3 layers, now identifiable as labrum, dorsal mandibular lip and ventral mandibular lip. In Figure 92, the fractured tip shows clearly that the labrum is filled with cellular material and the mandible retains a small amount also. This figure compares well with that of Figure 80, which shows a trapezoidal, cell filled labrum inside the arcuate mandible. No observations suggest how the stylet inserts itself into the mandible.

The Pharate Larval Lycid Syntrophium

The operation of decapitating and fixing an apolytic, then extracting the head from its exuviae was exasperatingly difficult for several reasons. The tissue is very soft, yet snug in the surrounding exuviae. Considerable tugging and wiggling is necessary, especially to pull the pharynx over the crop. Ecdysing larva must also "unswallow" the whole stomodeal exuviae, but the crop probably collapses to allow smooth passage. In the case of my specimens, the fixed crop retains its girth. Also, there was no way to monitor development to

determine optimum moments to kill. This resulted in redundancy of many specimens, but several interesting observations could still be made (Figure 94, Figure 95).

All mouthpart components are accounted for as minute lobes. The labrum and mandible are coalesced as one lobe with two peaks, suggesting that they must segregate themselves again. The maxilla is conical and surprisingly short, the galea is globose and positioned at the base of the maxilla, and the labial palps appear as two tiny tubercles in their expected position. A fossa is conspicuous in the gular region.

It was not determined whether any cells were left behind in the exuvial syntrophium. Since the developmental cycle has not been staged, it is also not known whether the results in the figures represent the last stages in drawing back to a flat surface, the first stages in new growth from a flat surface, amputation back to stem cells, temporary withdrawal of cell populations into the head, and so on. Considering the extreme attenuation of cells in the interior of the thin labrum and mandible, it is possible that they and their corresponding lobes are equivalent in volume. The short maxilla is perplexing; there is clearly some loss of volume in the process.

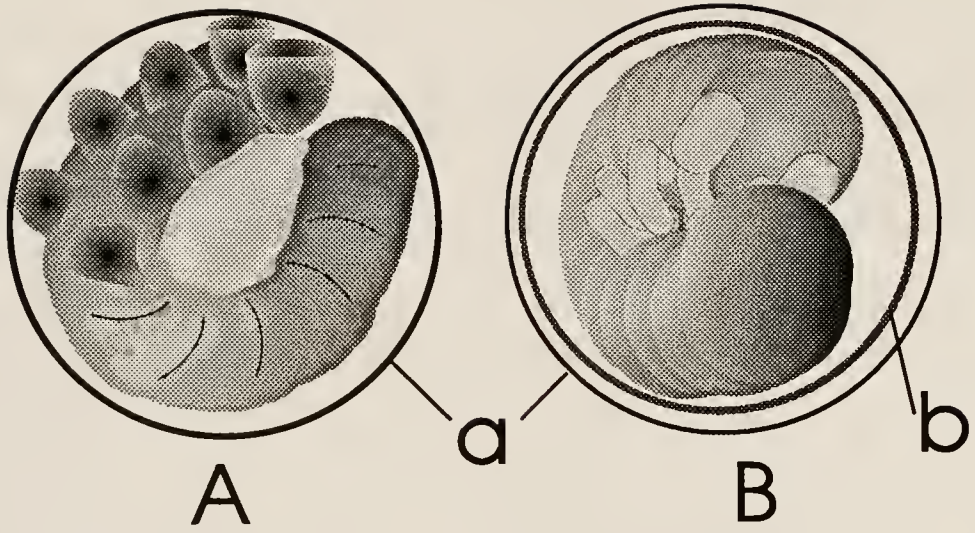
The procedure was not carried further in pursuit of these questions since their relevance to the thesis at hand was limited. Regeneration of the pharate larval instar remains a very fertile area of research in developmental biology.

The Pharate Tenebrio Larval Mandible

Results showed a second novel and curious pursuit. The mandible exuviae can be pulled off the head like a dental crown off its root to reveal the regenerating mandible within (Figure 96, Figure 97). If kept moist and bathed with molting fluid, it might be possible to observe the whole regeneration process in vivo. The mandible is considerably smaller than the available space in the interior of the exuviae indicating that a drawing back of length occurred. There is no indication of a composite nature in cross-sections; cells assume a circumferential, crenate pattern and do not follow any invaginating planes or other infrastructure (Figure 98). As with pharate lycids, exploratory study showed the relevance of the procedure to the thesis at hand to be highly limited and so it was not carried further.

Figure 33. Calopteron mutants. A) Failure to undergo katatrepsis. B) Failure to breach the serosa during katatrepsis. a) Chorion. b) Serosa.

Figure 34. Photuris eggs dried to about an 80% loss of spherical volume. Larvae within suffer extreme contortion from strong, localized, pressures.



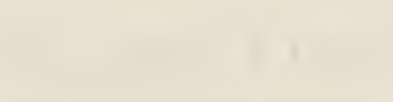


Figure 35. First in longitudinal series, from dorsum to venter. Canal actually opens dorsally to the buccal cavity. The section angle makes it appear to open laterally. See Figure 42. A) Dorsal deflection. B) Antenna.

Figure 36. Second in long series. A) Amorphous areas. B) Apparent seam is probably a setal socket as no such seam is indicated in transverse sectioning.



Figure 37. Third in long series. A) Section grazes retinaculum.

Figure 38. Final in long series. A) Section grazes tip. B) Stomodeal exuviae. C) Base is expanded interolaterally.

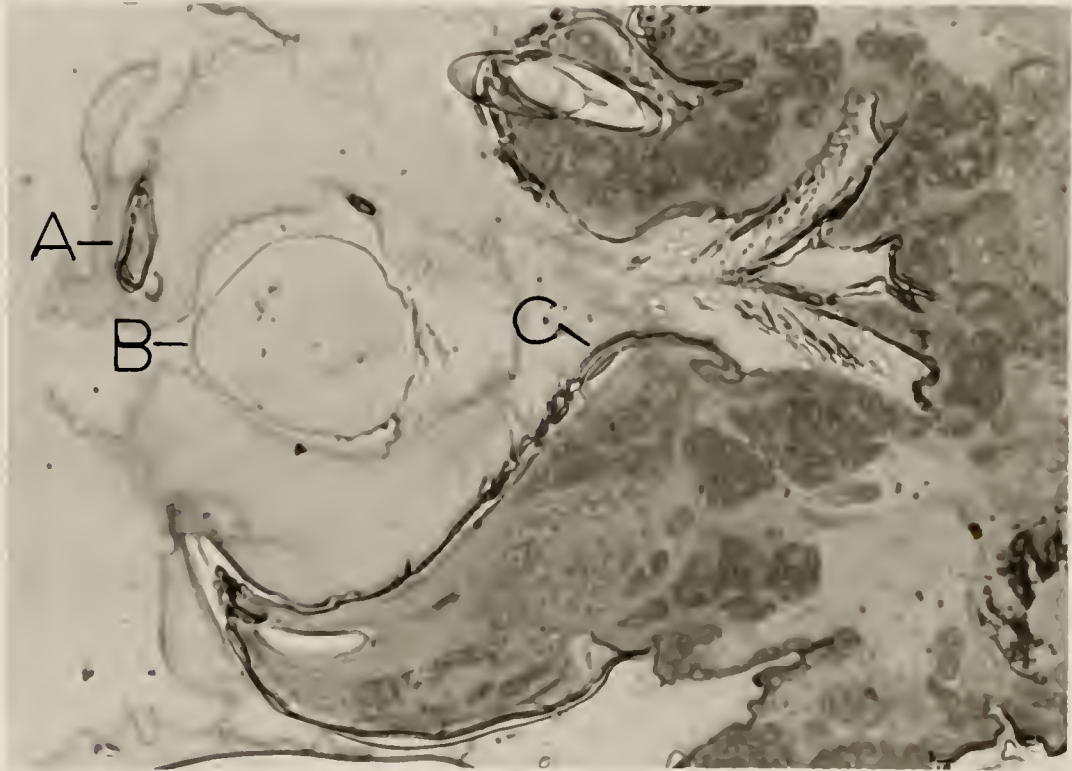
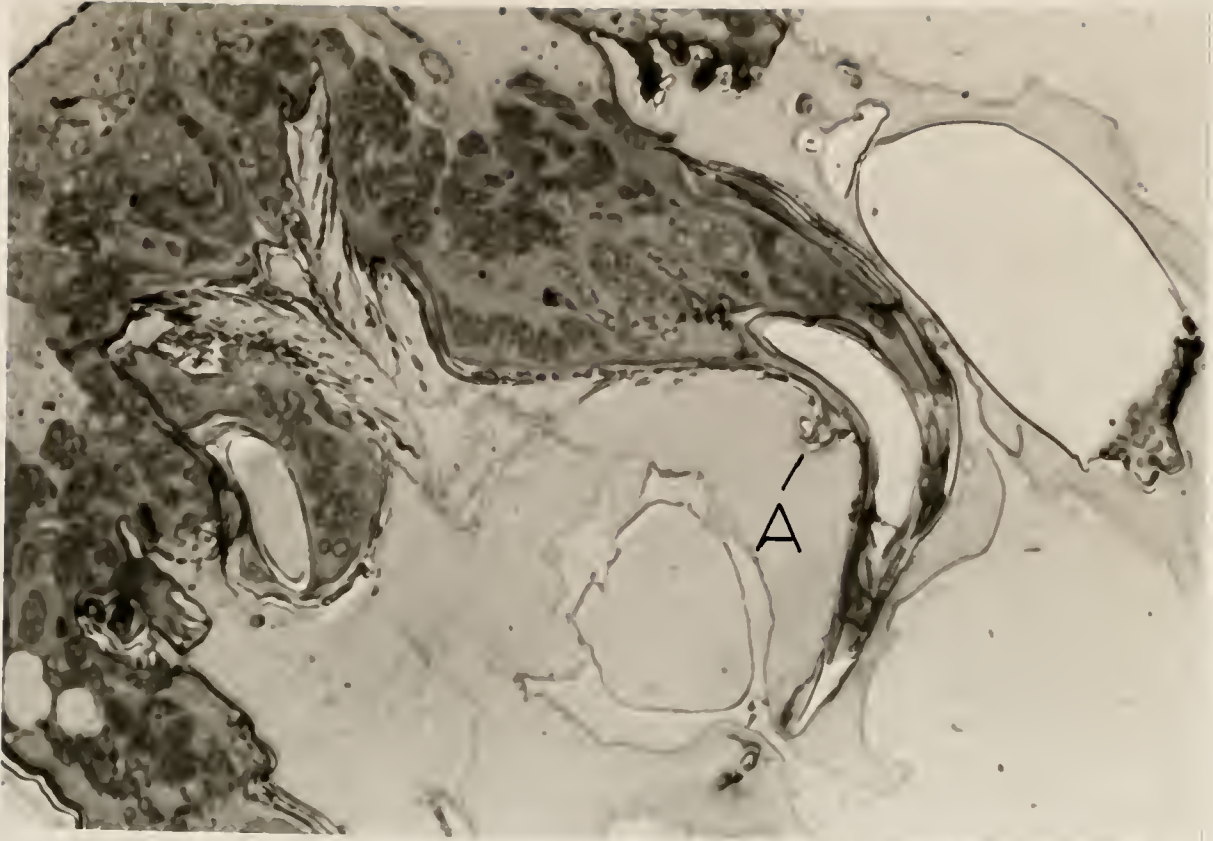
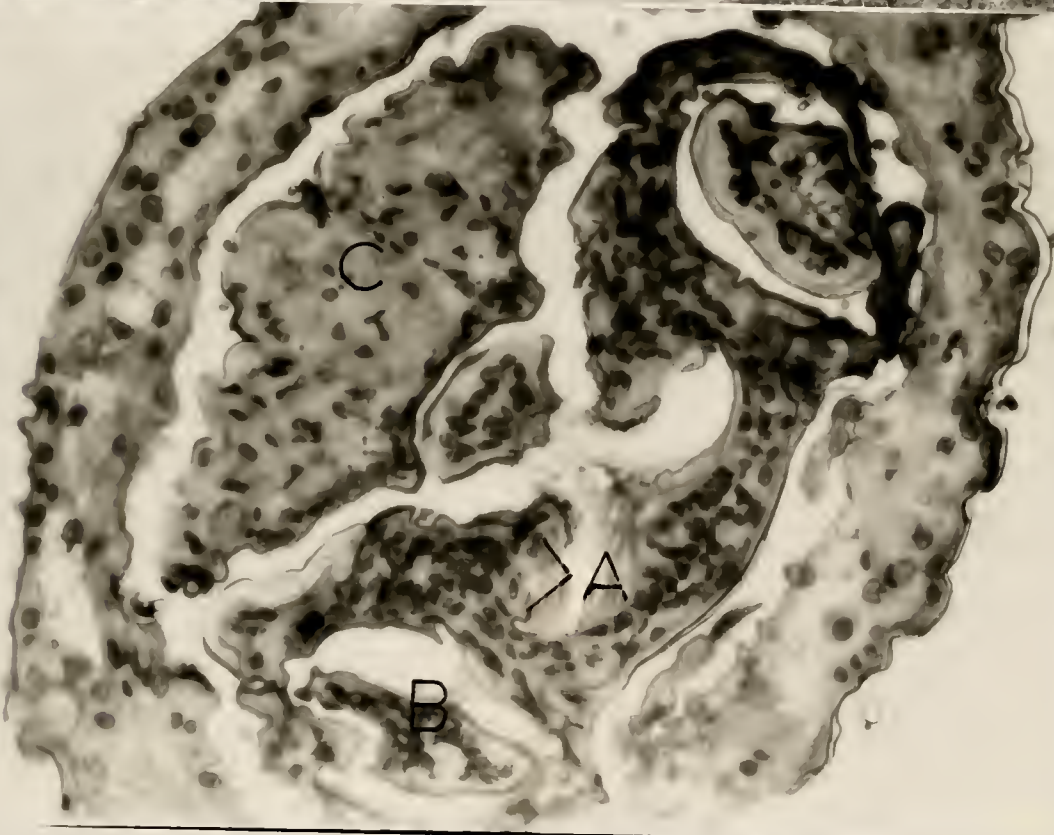
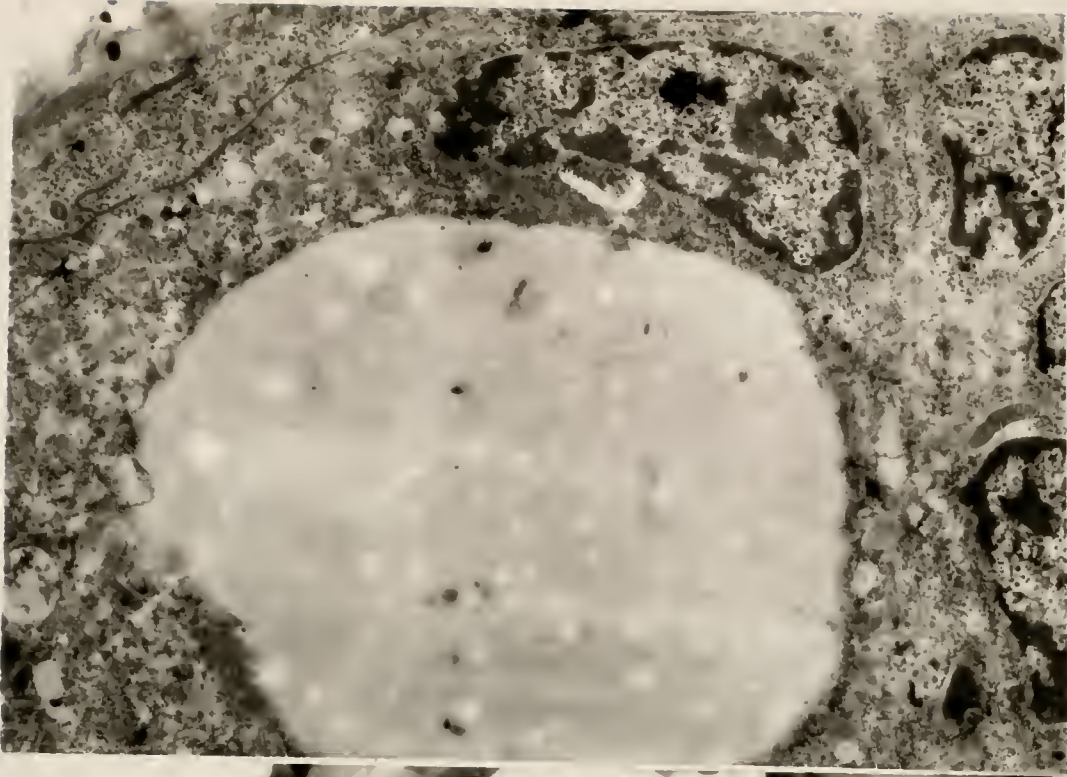


Figure 39. Close up of amorphous disc in Figure 36A.

Figure 40. First in transverse series of P. collustrans, from base to apex. A) Arrows point to the two lobes that are under observation. B) Antenna. C) Maxillar tissue.



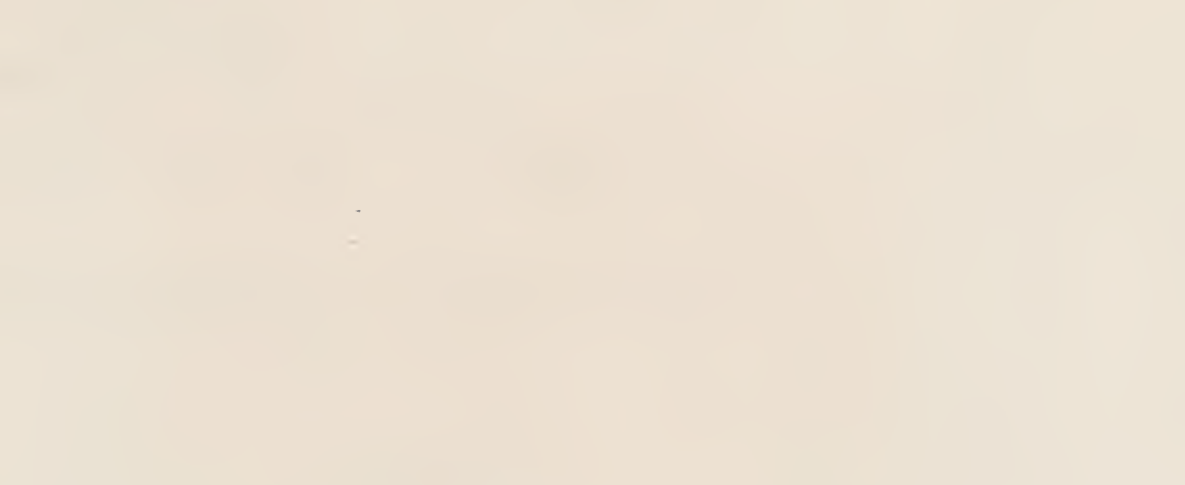


Figure 41. Second in transverse series. A) Continuity from transfrontal region to syntrophium.

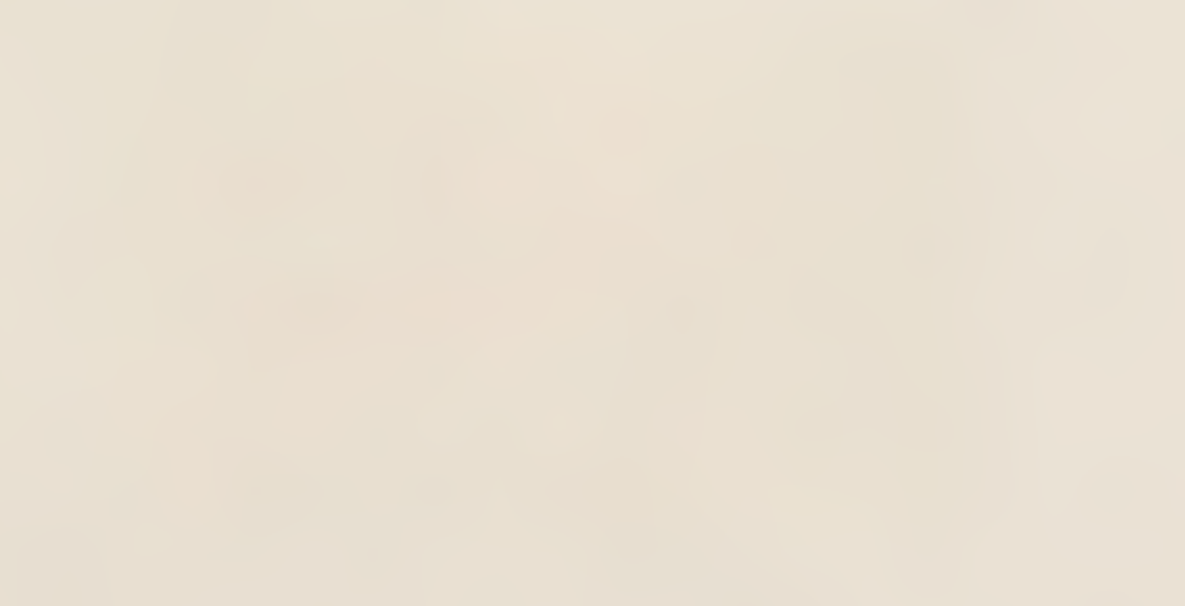


Figure 42. Third in transverse series. Dorsal closure is approached. A) Cells back away from the apex of the 2 lobes to form caps of cuticle.

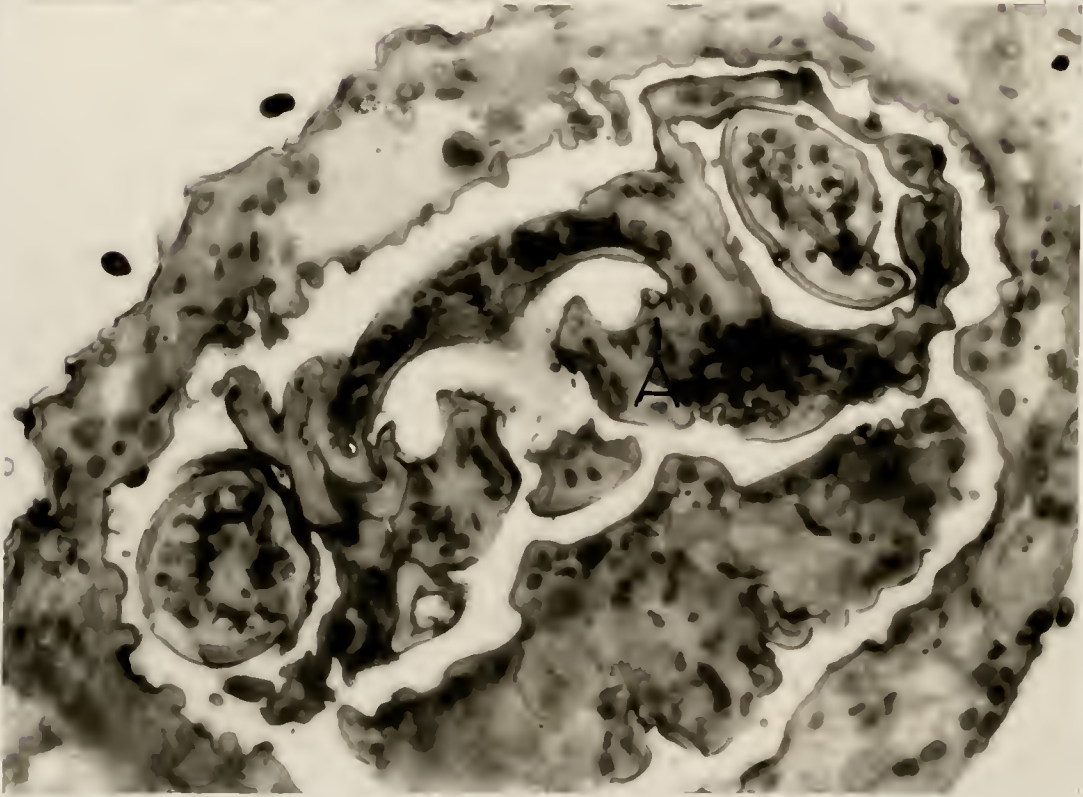
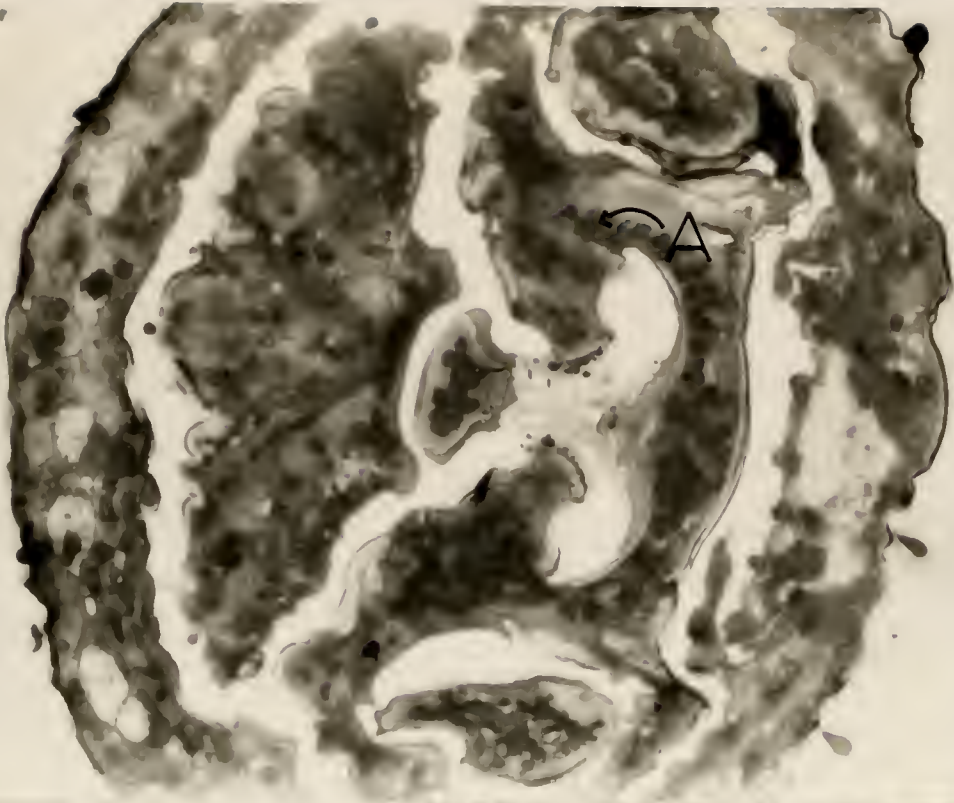


Figure 43. Fourth in transverse series. Dorsal closure of the canal is reached. A) Canal lumen is closed to the mouth at this point.

Figure 44. Fifth in transverse series. A) Horse-shoe shape of cuticle. B) Area continuous with head tissue is outside the embrace of the horse-shoe.

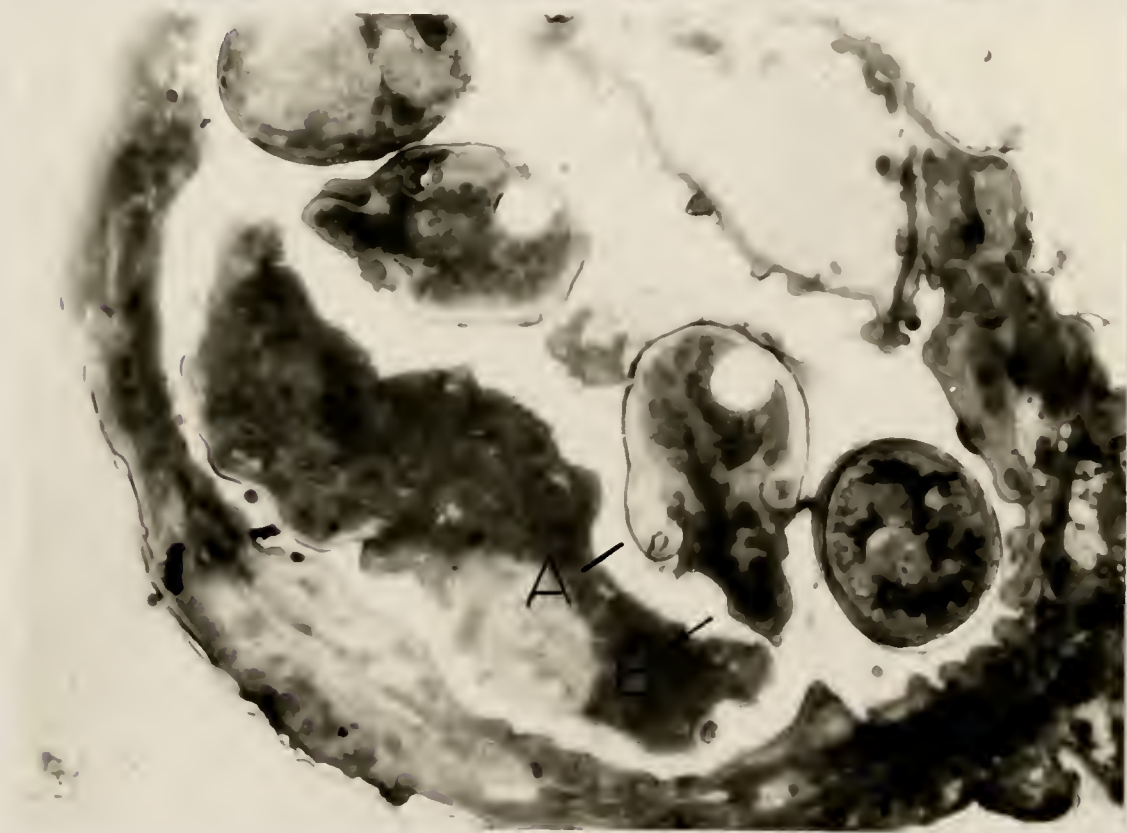
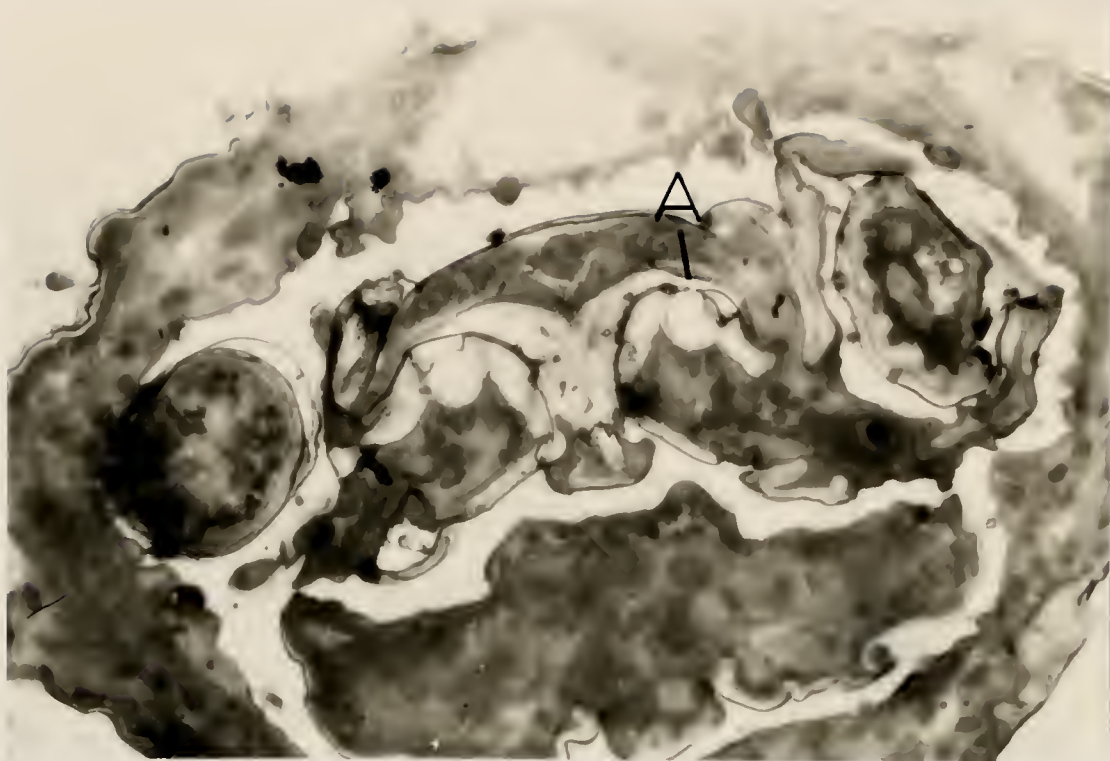


Figure 45. Sixth in transverse section. A) Points to a lateral area that deposits a welt of cuticle of its own between B) and C), the ends of the horse-shoe.

Figure 46. Final in transverse series. Section cuts tangent to the pore. Below are labial palps, gala and maxillary palpifer.

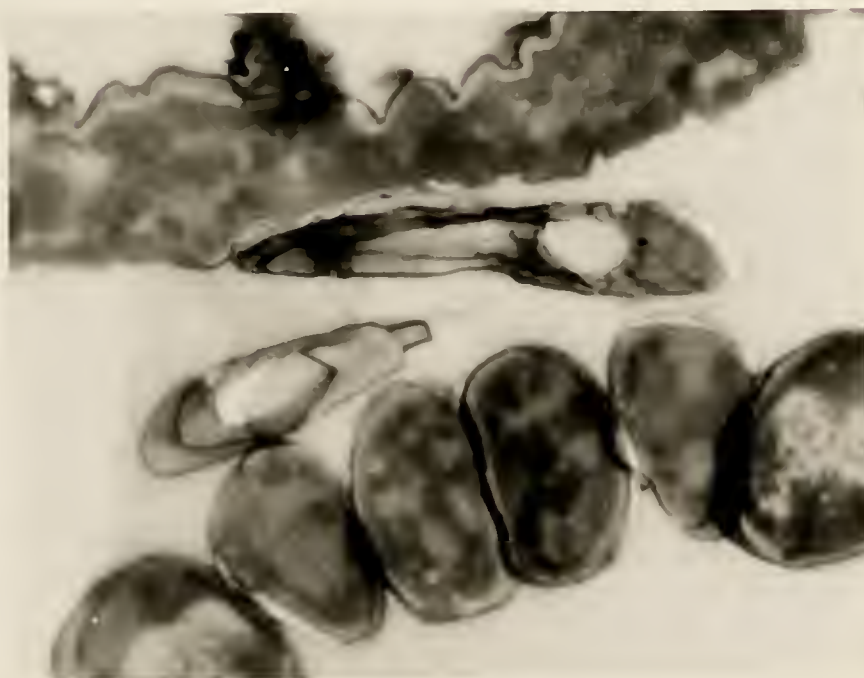


Figure 47. SEM of Photuris 1st instar showing pore, upwardly angled tips and retinaculum, the latter partially hidden by setae.

Figure 48. Pore of Pyractomena larval mandible. A) Although partly obscured by foreign material, a seam can be seen to extend apically for a short distance.

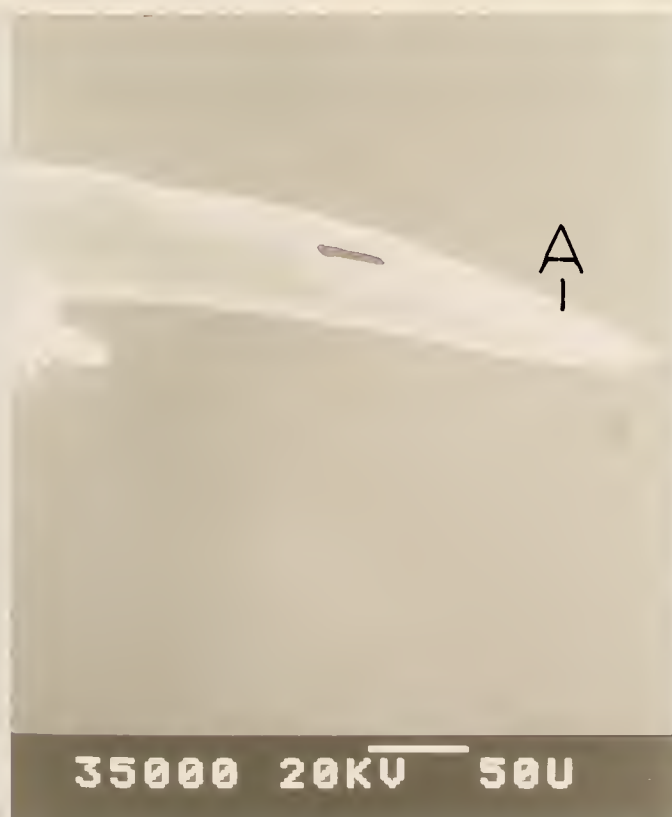


Figure 49. Long section through the tip of a P. collustrans mandible. Cell diameter is extremely attenuated.

Figure 50. Cross-section through extremely tough tissue forming the apex of a P. collustrans mandible. A) Origin of a seta at the end of a dendrite.

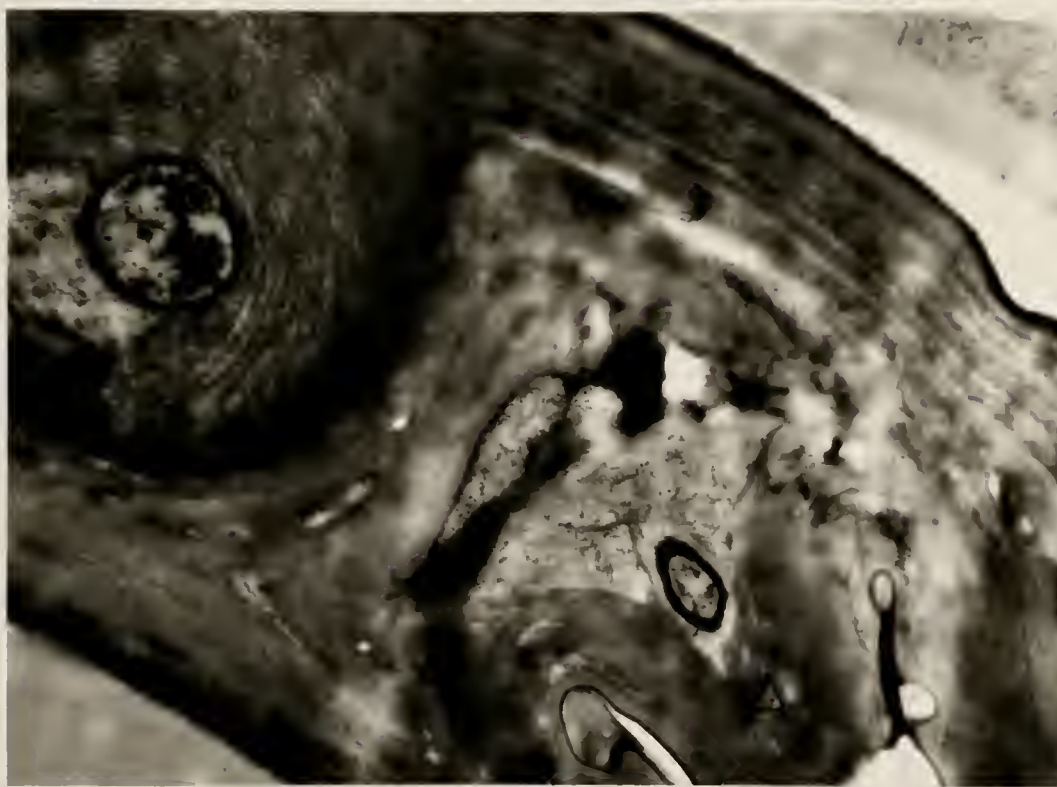


Figure 51. Innervation of the mandible tip in P. collustrans.
A) Dendrite. B) Canal lumen.

Figure 52. Tip of P. collustrans mandibles. A) A branched seta at the pore. B) Lack of a seam. C) A campaniform sensilla is on the opposite side.

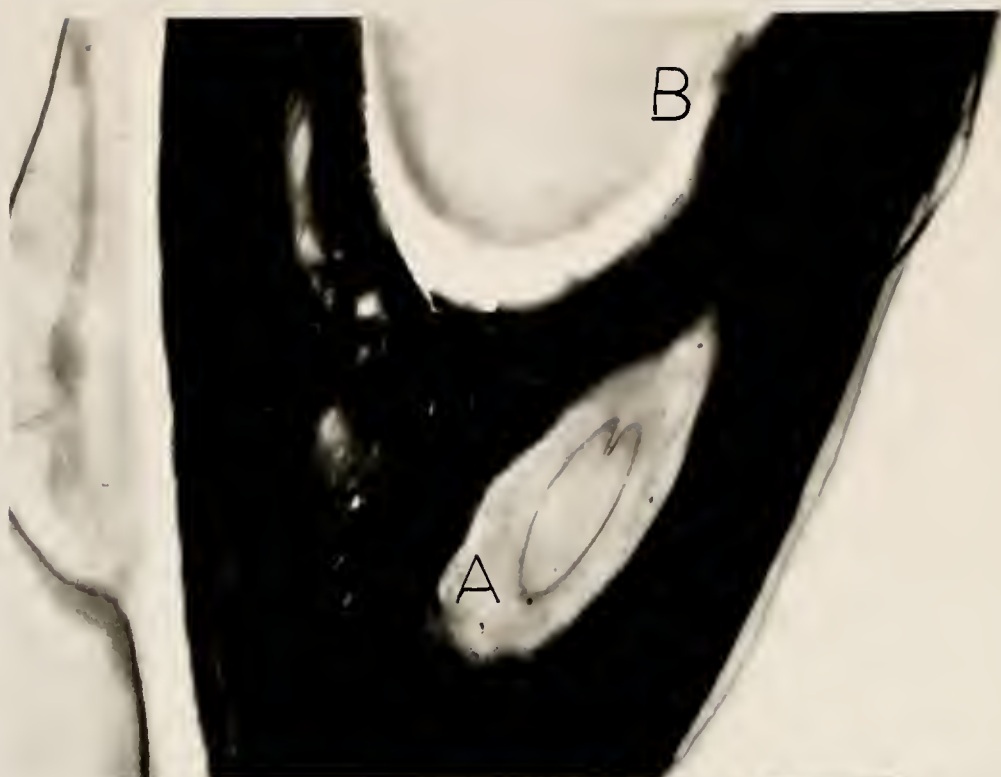
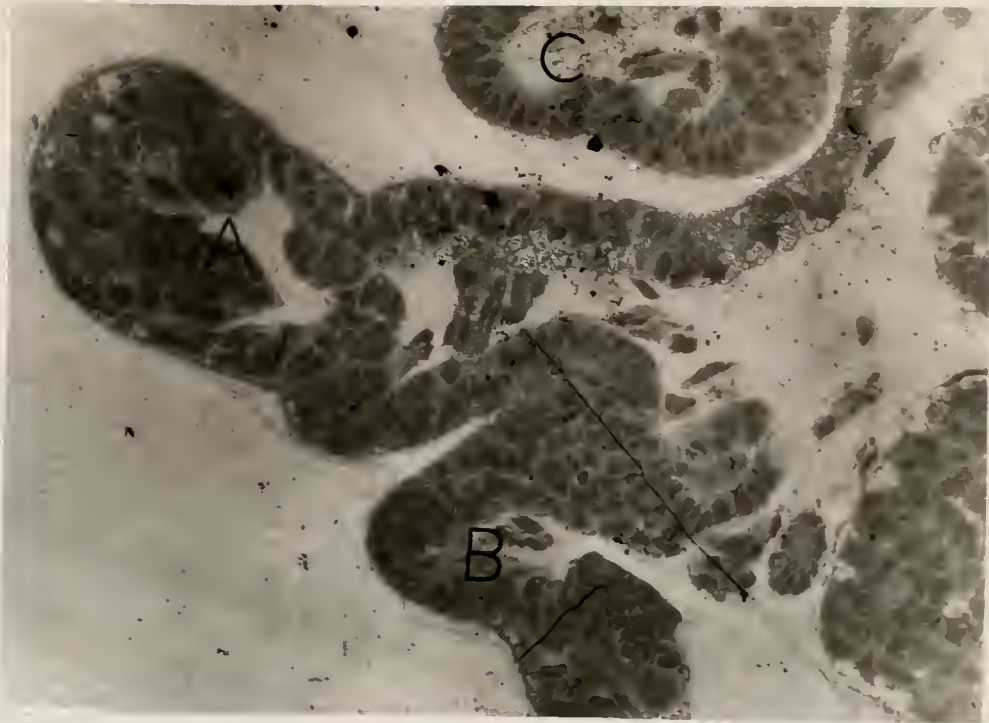


Figure 53. First in series of an anatreptic P. collustrans head capsule. A) Elongate lobe. B) Smaller lobes. C) More lobes occur above (A); these are shown in Figure 54. There is no indication of which lobe corresponds to which mouthpart.

Figure 54. Same section as Figure 53. A) Elongate lobe, same as Figure 53A. B) Shorter lobes pointed to in Figure 53C. There is no definitive landmark for orientation.



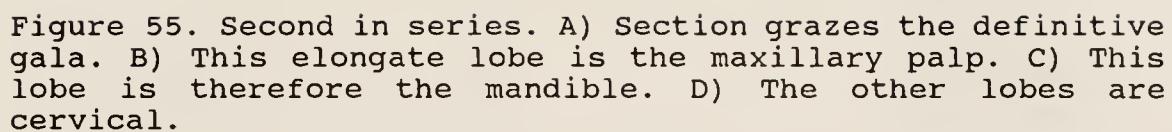


Figure 55. Second in series. A) Section grazes the definitive gala. B) This elongate lobe is the maxillary palp. C) This lobe is therefore the mandible. D) The other lobes are cervical.

Figure 56. Final in series. A) The gala protrudes distinctly.

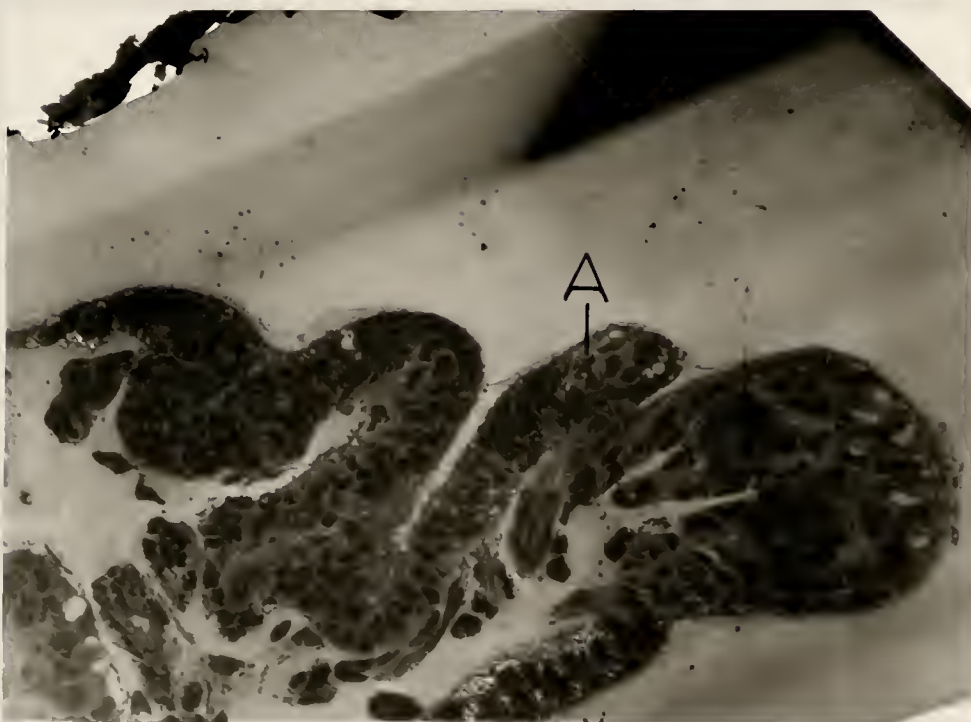
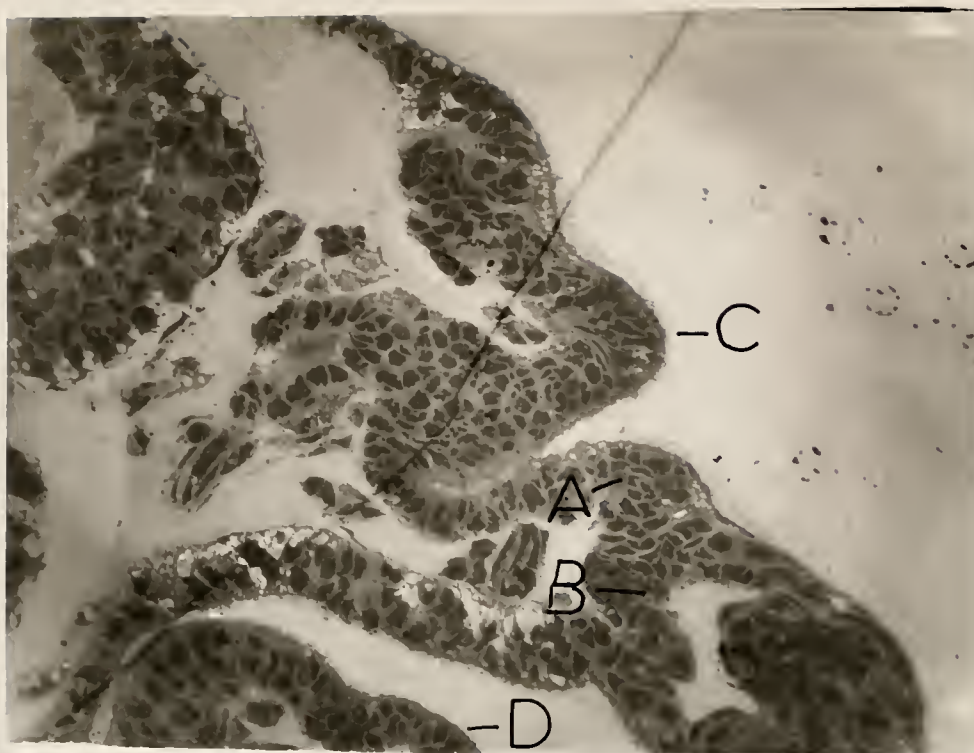


Figure 57. Young mandible, prior to commencement of curvature, showing the apical invagination. Also shown by SEM in Figure 32. The 3-armed pattern at the palp apex is a peculiar ripple in the section.

Figure 58. Young lampyrid syntrophium at commencement of elongation and curvature. Note the extremely high nuclear: cytoplasmic volume ratio.

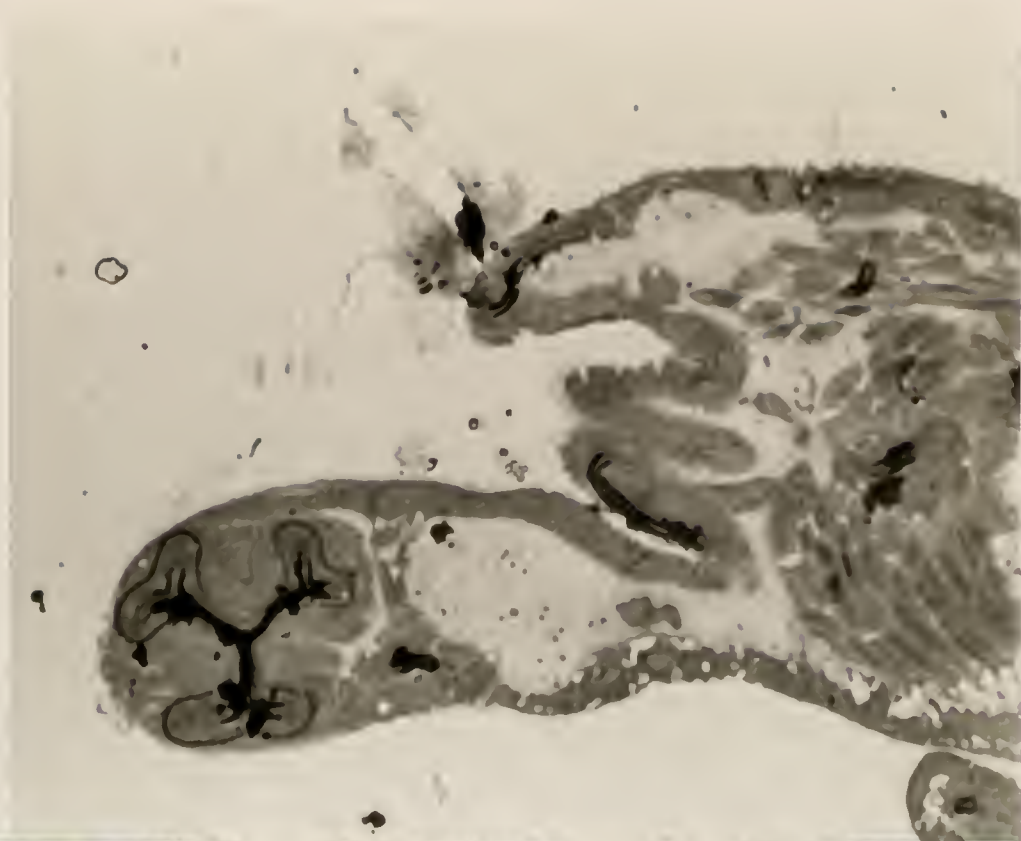


Figure 59. Young P. collustrans fixed and embedded at a point when the mandibles are first undergoing curvature. A) Stomodeal exuviae.

Figure 60. Long section through the dorsal deflection. A) Incipience of the canal.

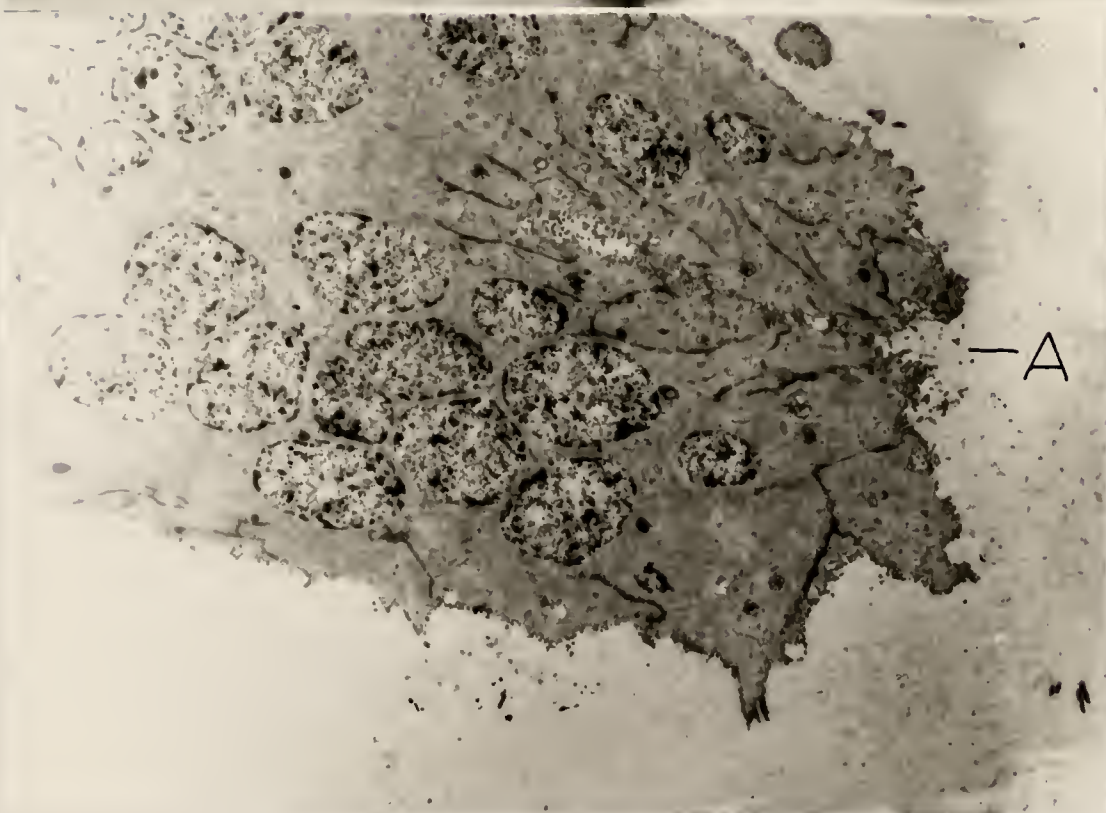
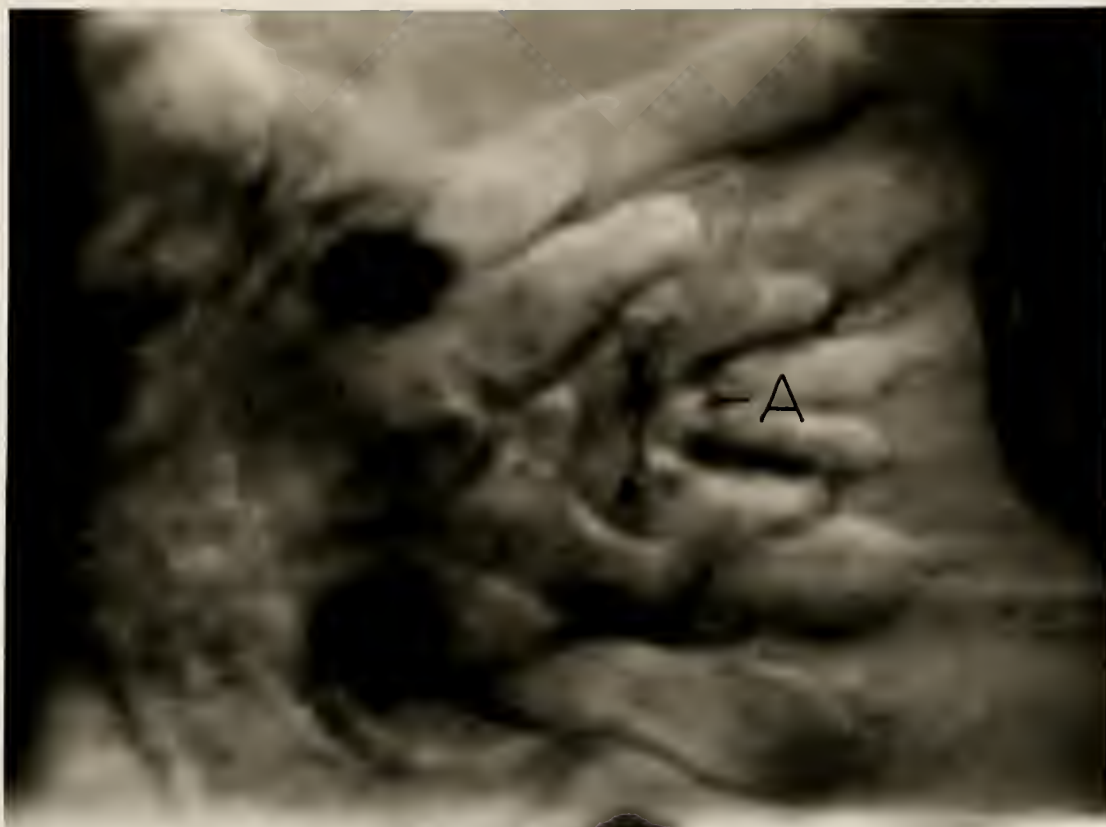


Figure 61. Facsimile 3-D reconstruction of the P. collustrans mandible. Reconstruction ascertains which of two grazed areas, circles, is of interest. Square shows where Figure 60 is located. A) Incipience of canal. B) Dorsal deflection.



4

Figure 62. Montage of specimen in Figure 60. A) Canal orifice.

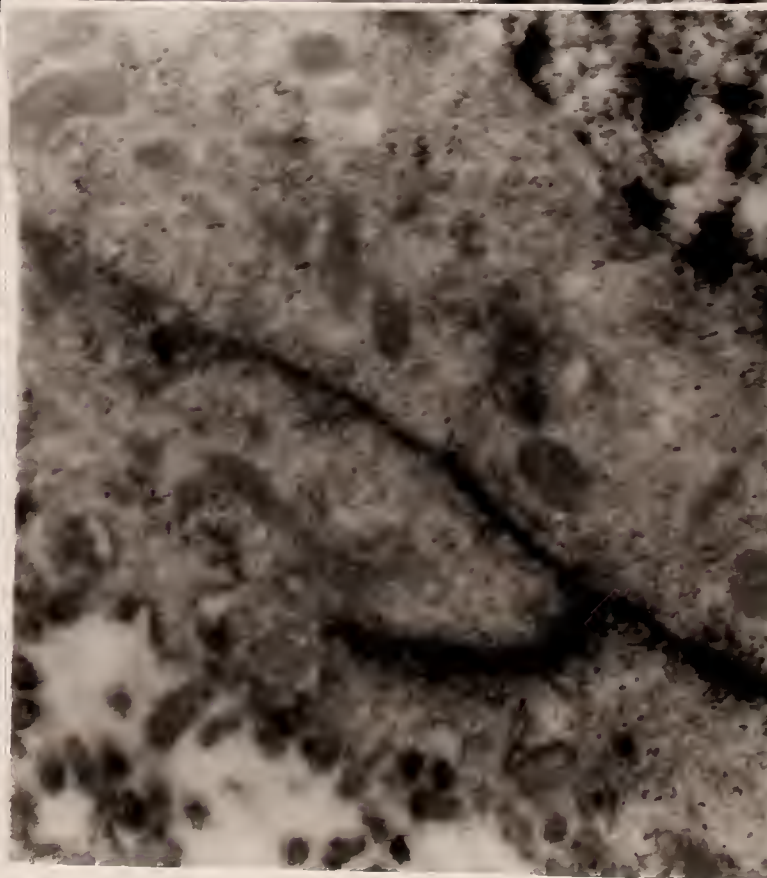
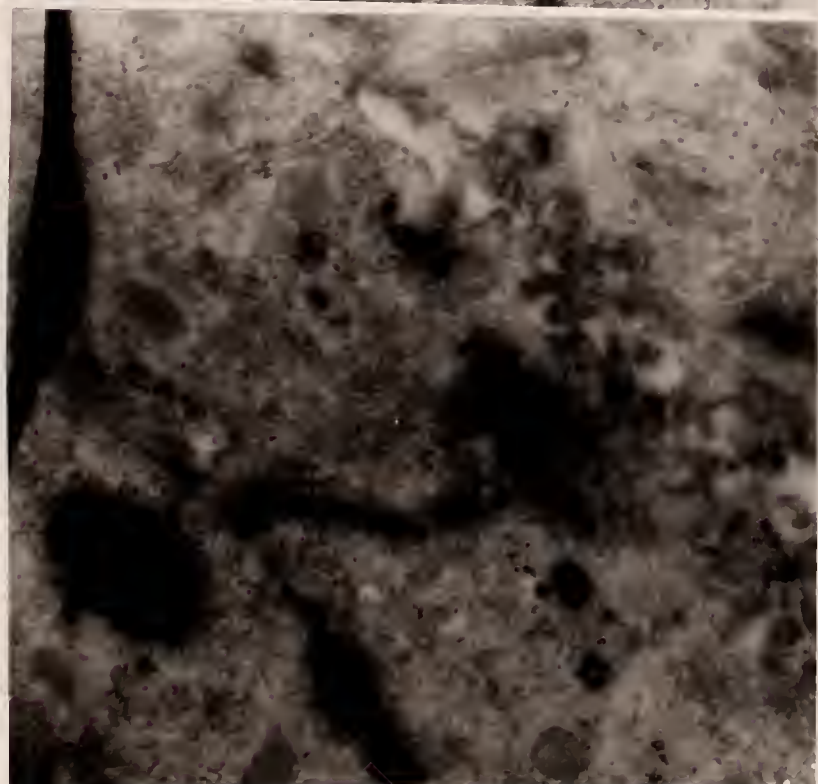
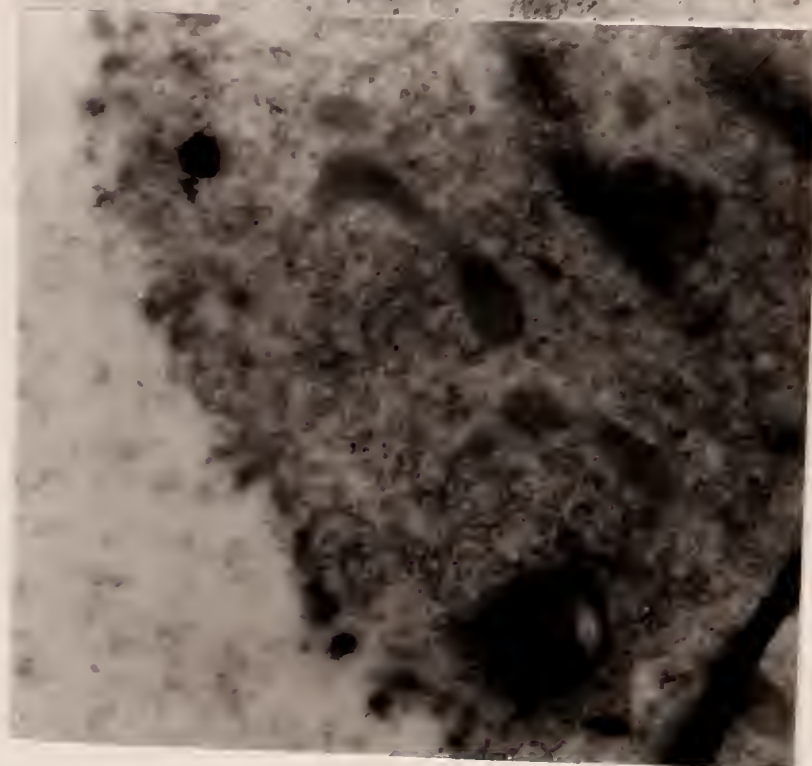
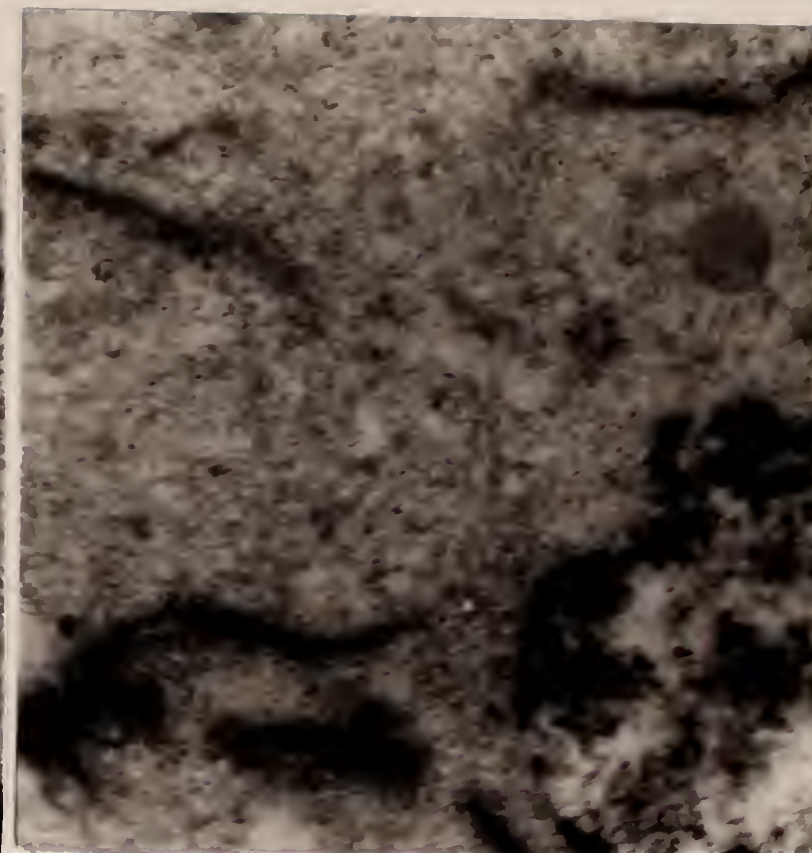
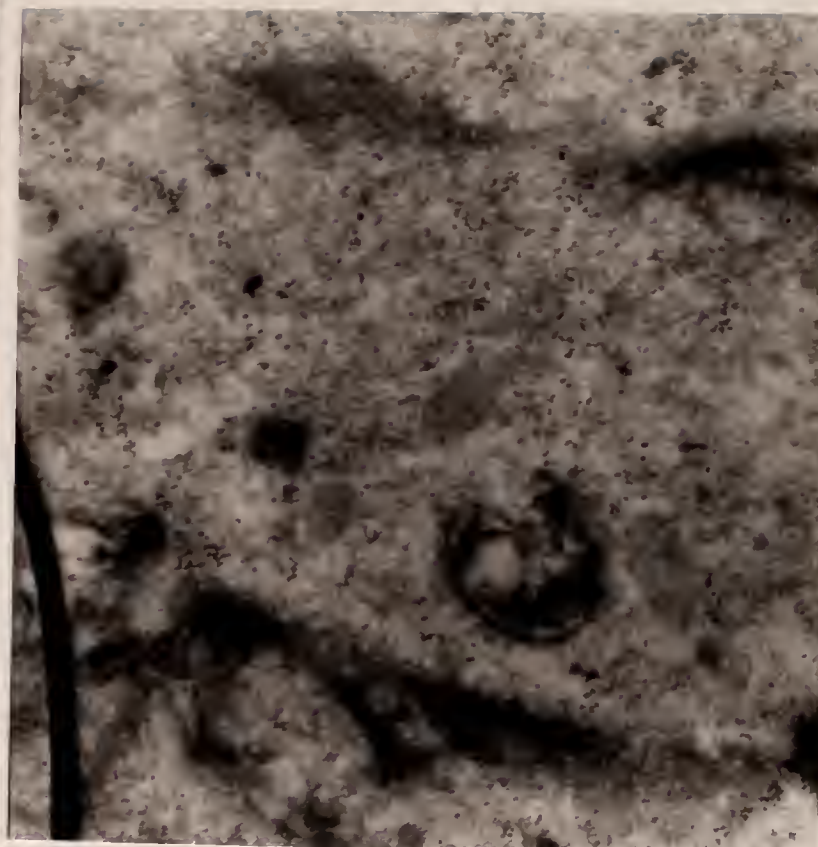
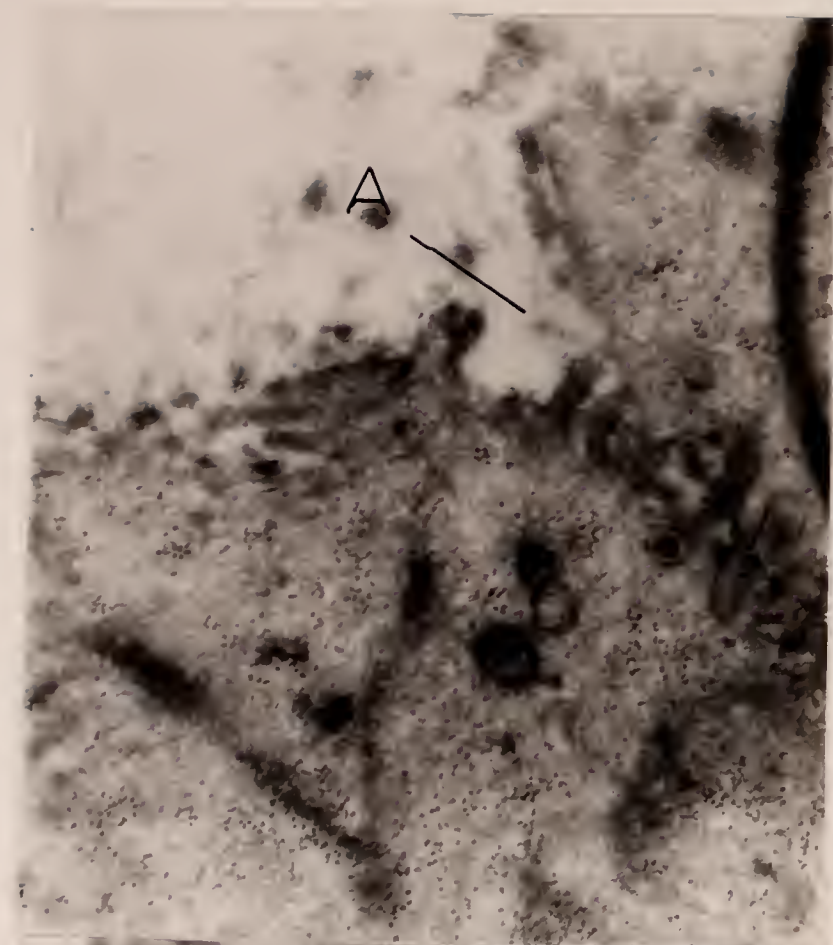


Figure 63. First in longitudinal series of a P. collustrans katatreptic syntrophium after curvature has commenced. A) Section grazes midlength of the mandible, showing that the canal has progressed at least to this point.

Figure 64. Second in long series. A) Origin of the canal. B) Progress of formation is at least to near apex.

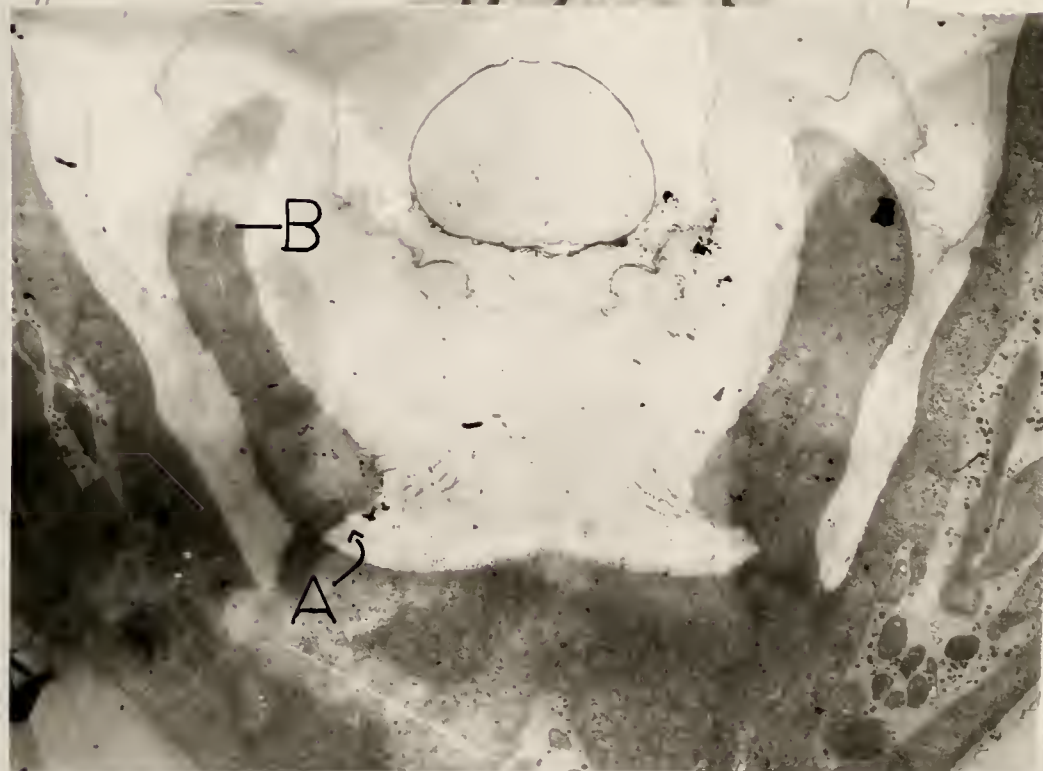
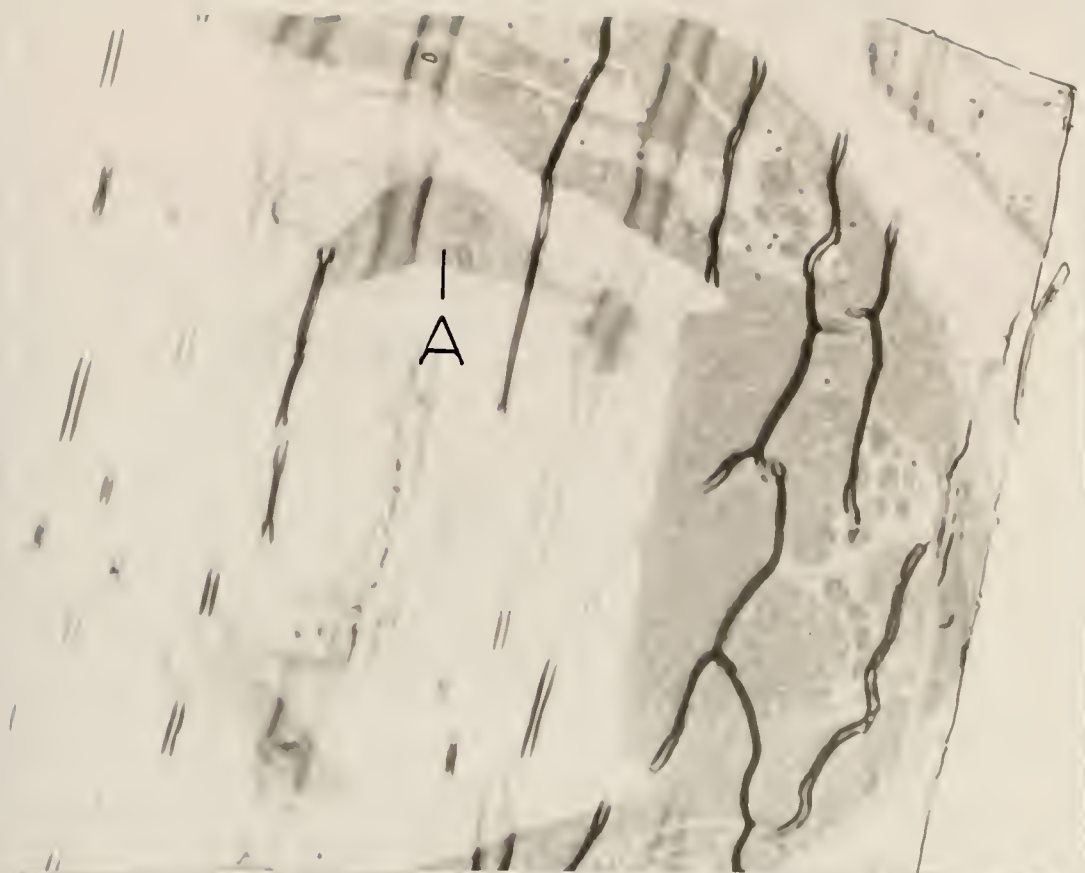


Figure 65. High magnification of a region in Figure 64, showing the microvilli that appear during canal formation.

Figure 66. Third in long series. Section passes beneath the canal and the longest length. A) Conduits are present that are probably haemocoelic. See Figure 67.

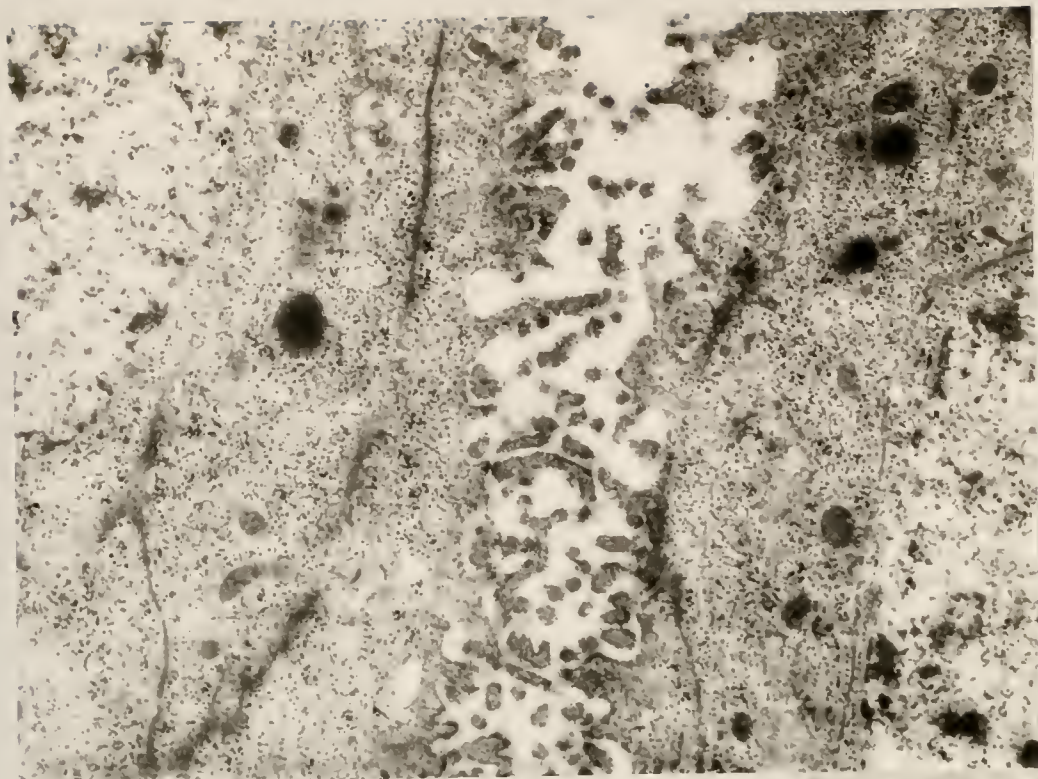
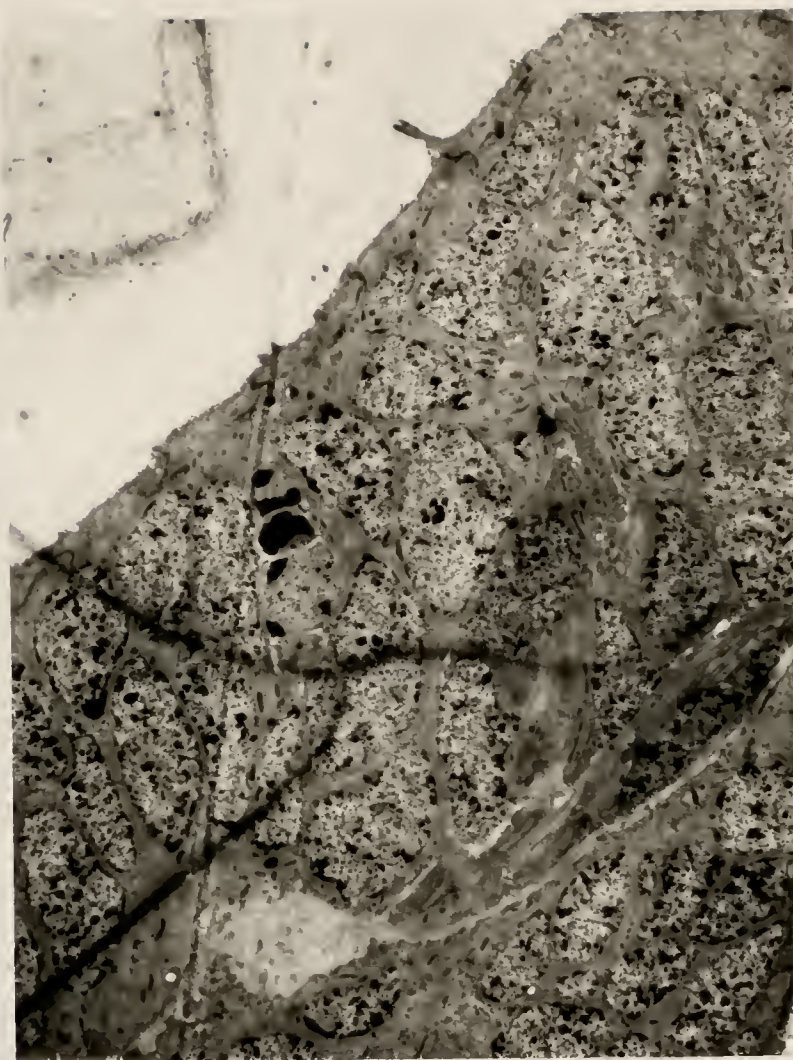


Figure 67. High magnification of the apex in Figure 66, showing presumed haemocoelic conduits.



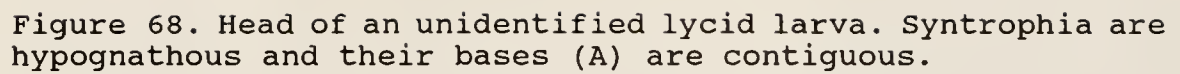


Figure 68. Head of an unidentified lycid larva. Syntrophia are hypognathous and their bases (A) are contiguous.

Figure 69. Interior view of head exuvial halves opened and laid flat as in Figure 8. A) Acetabulum. B) Ridge. See Figure 90. C) Crop.



Figure 70. First in an extensive cross-sectional series of Calopteron first instar. A) Labrum is continuous across front. B) Its venter infiltrates the arc-shaped mandible.

Figure 71. Second in series. A) Section grazes the anterior extent of the labrum.

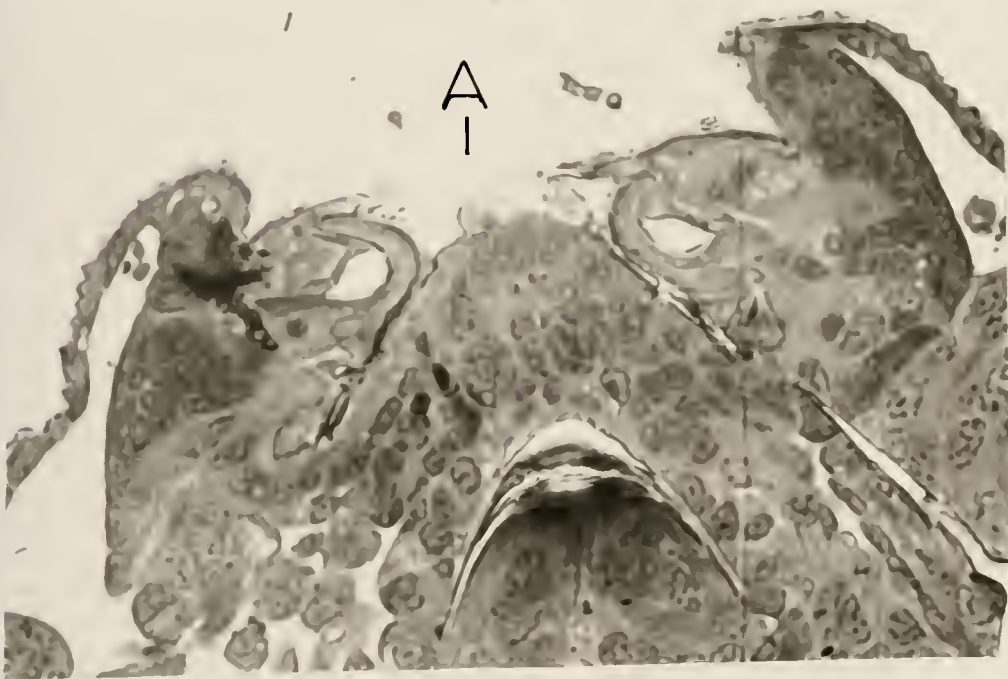
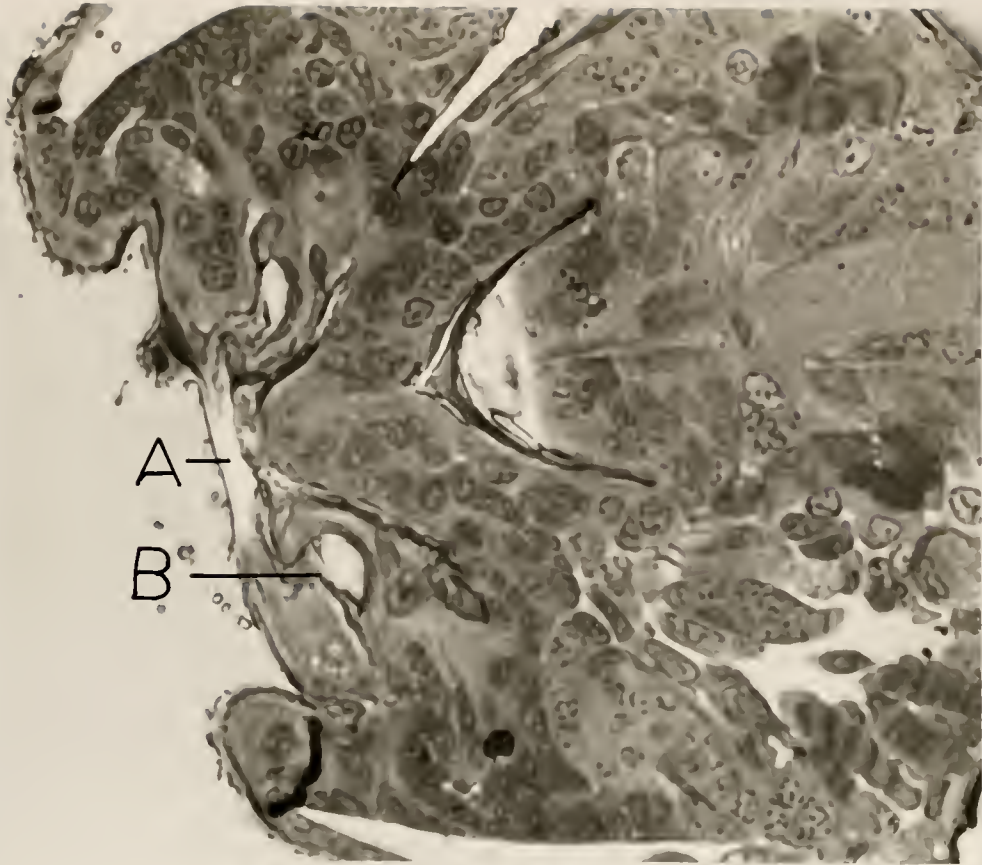


Figure 72. Third in series. A) Labral halves are separate and, B) fully contained within the arc-shaped mandible.

Figure 73. Closeup of the base in Figure 72. A) Nerve bundle is separate from the labral wall at this cross-section. Black spheres are post-stain residue. B) Labromandibular junction.

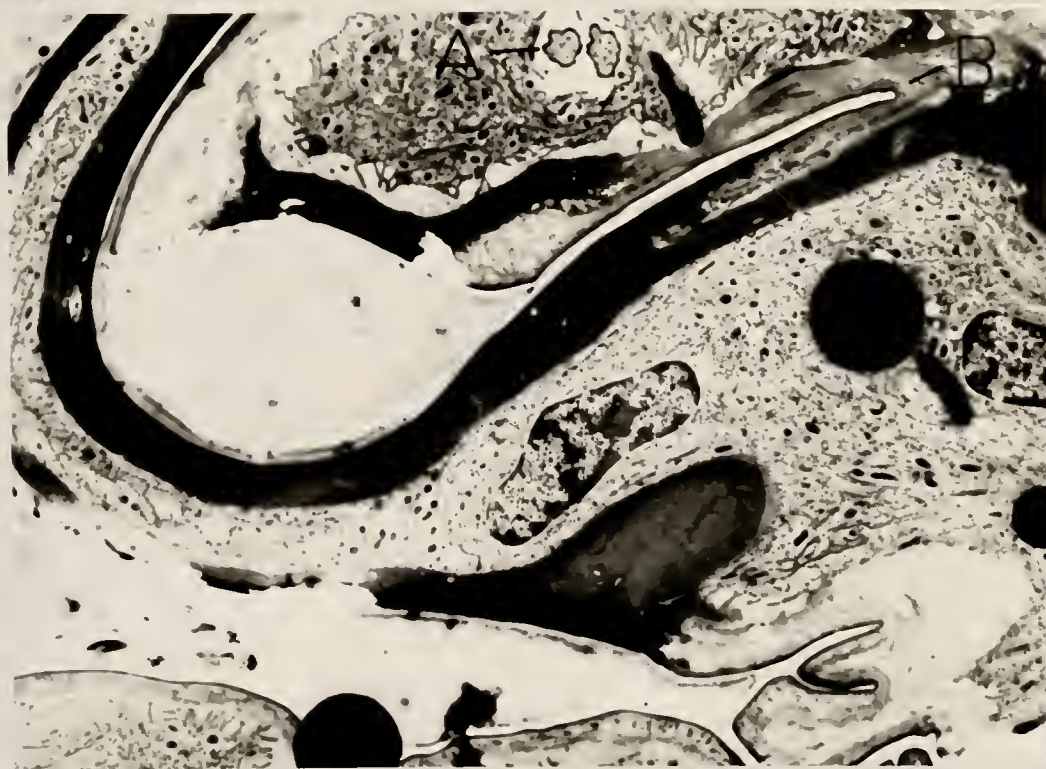


Figure 74. Sectioning continues. A) Gala is reached.

Figure 75. Close-up of gala-labromandibular interface.

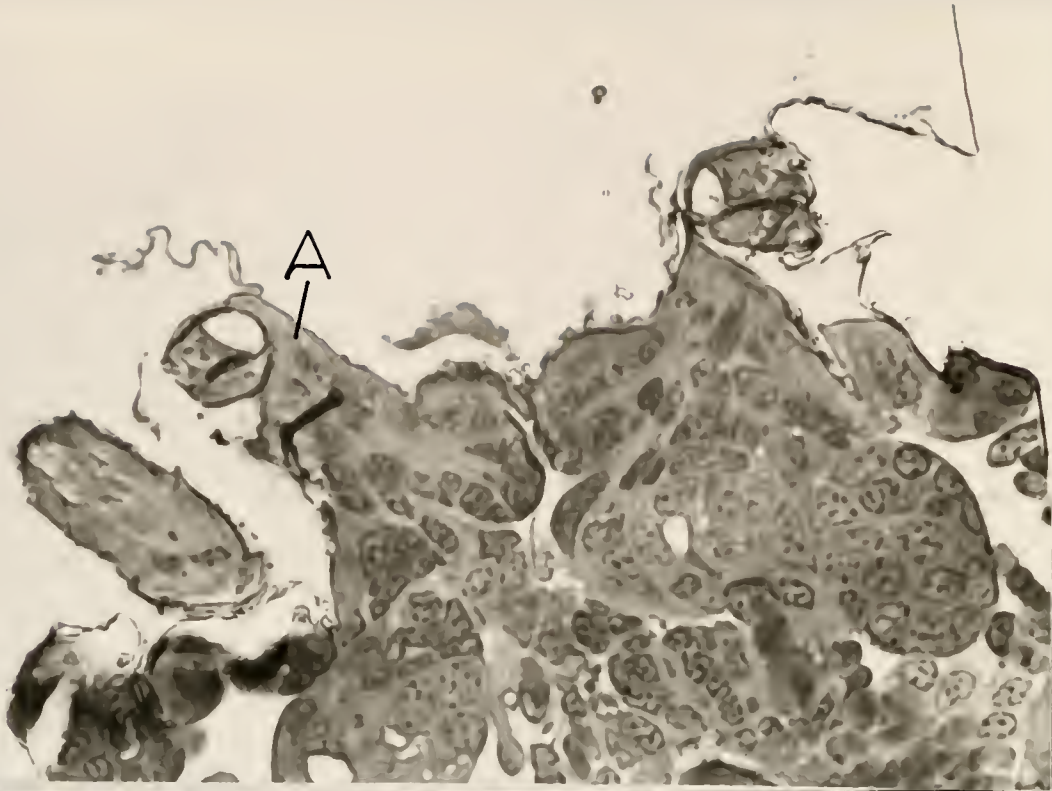


Figure 76. Extreme close-up of Figure 75. Gala is independent.

Figure 77. Closeup of nerve bundle in Figure 75. A) Fibrils.

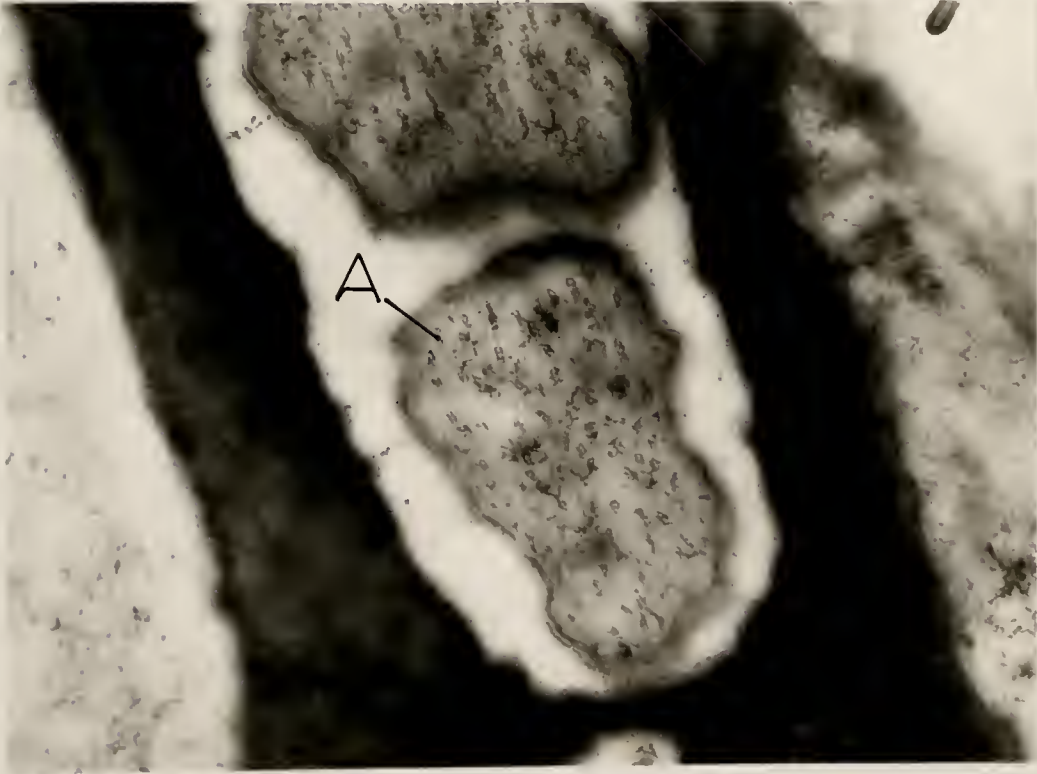
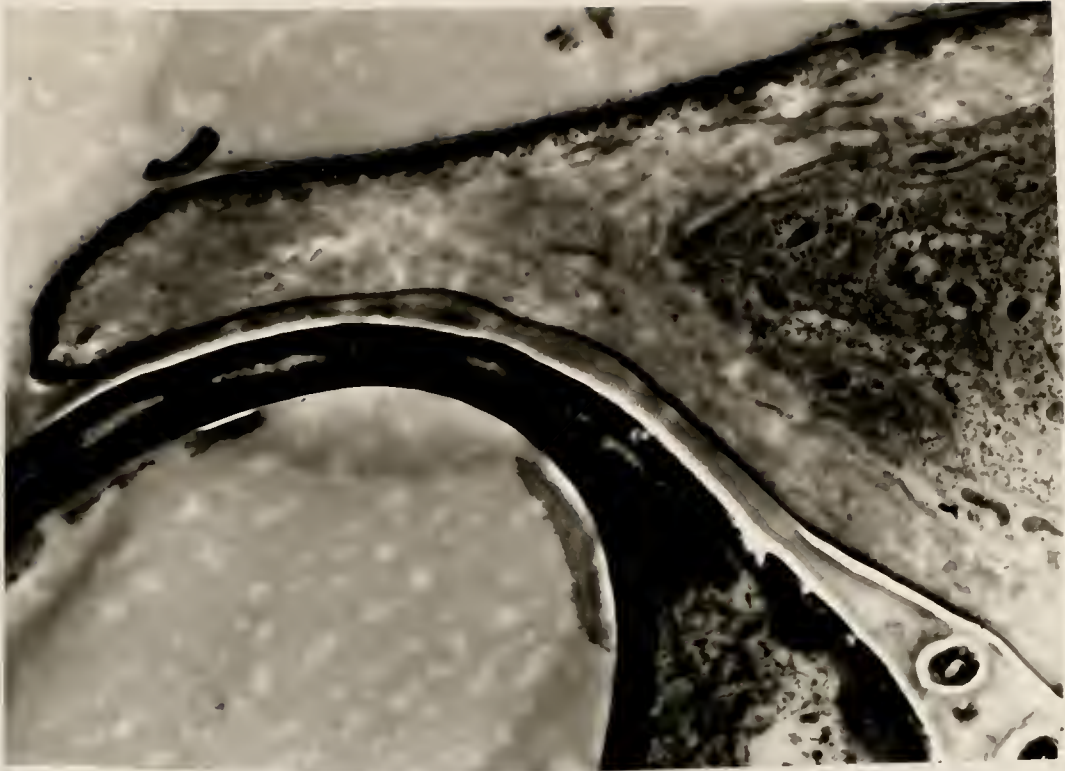


Figure 78. Sectioning continues. A) Gala ensheathes. B) Maxillary palpifer appears.

Figure 79. Closeup of left syntrophium in Figure 78. A) Center of the mandible. The canal tracks along this longitudinal.

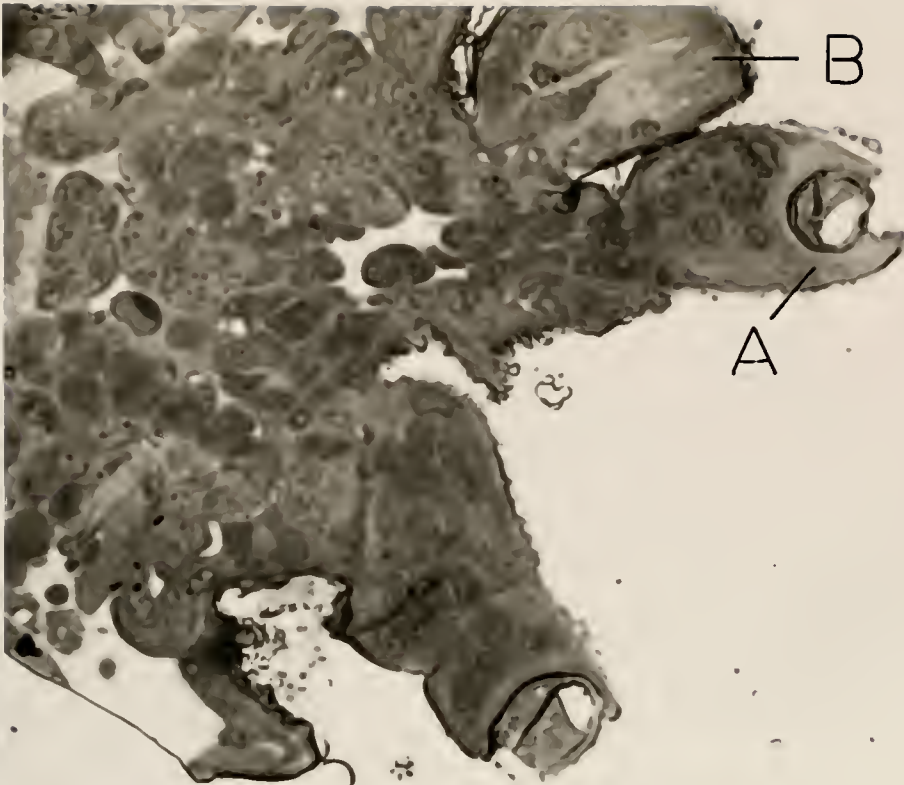


Figure 80. Final in series. Closeup of right syntrophium in Figure 78.

Figure 81. First in series of Lycus lecontei syntrophium apex. Mandible (lower) and gala (upper) exceed the labrum in length. The gala undergoes dramatic histological change from a cell-filled to, Figure 82, an alveolate interior.

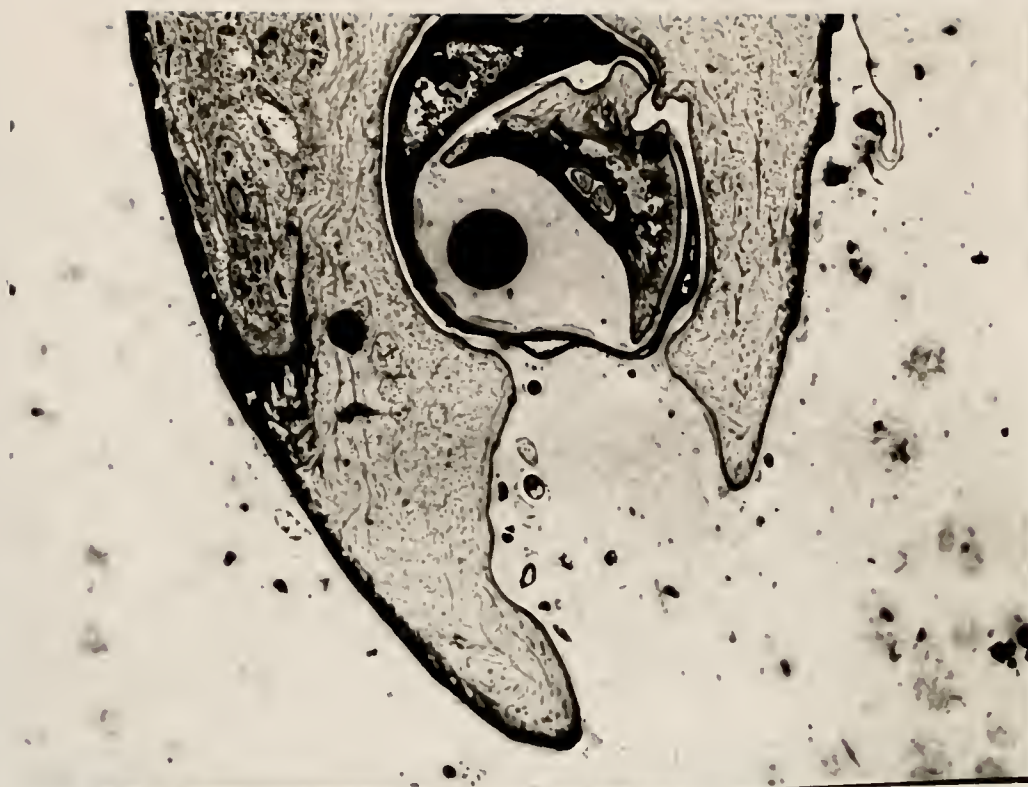


Figure 82. Second in series. The alveolate interior of the galar apex is formed by dehiscent spheres and probably functions as a sieve during feeding.

Figure 83. Final and distal-most cross-section of syntrophium. Mandible exceeds the length of the gala.

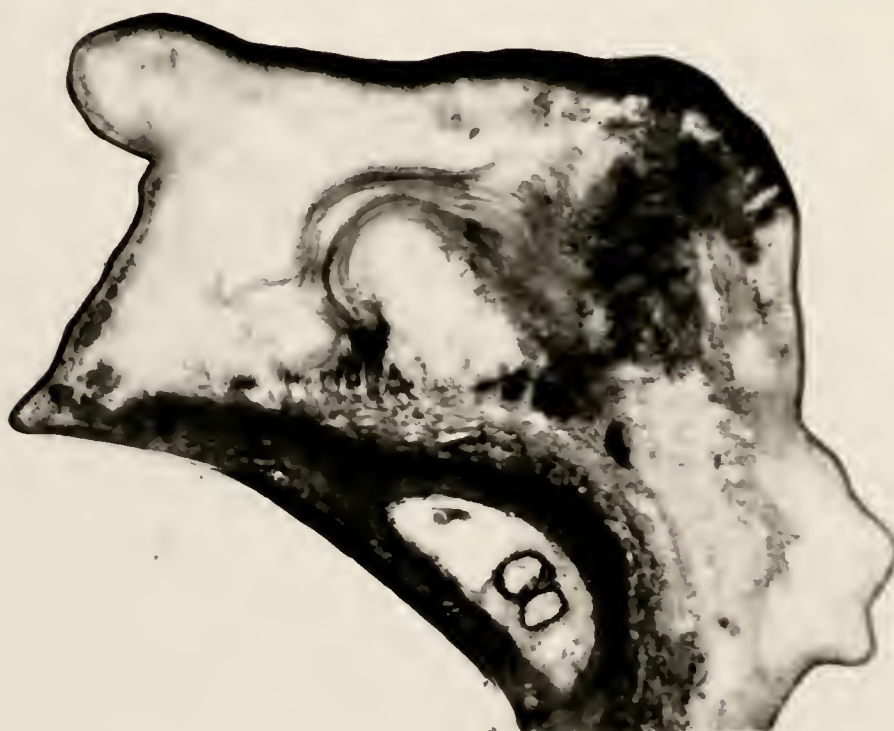
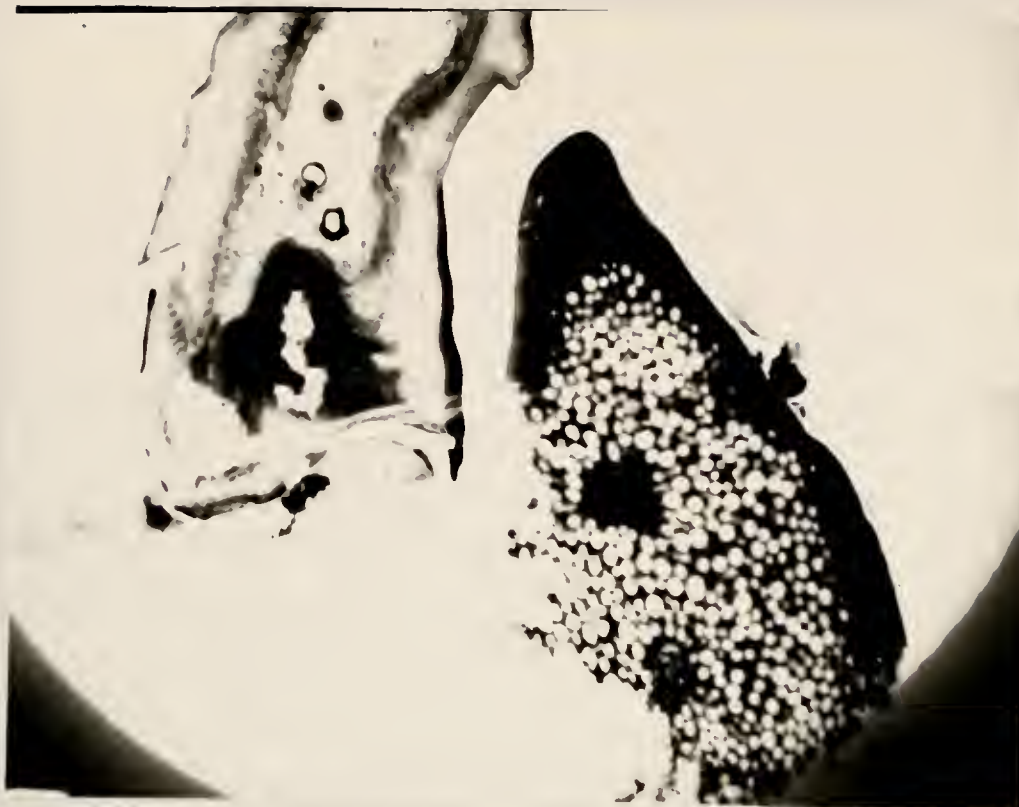


Figure 84. Exuviae of L. sanguineus stretched over a pinhead so that the mouthparts protrude. Figure 85 through Figure 87 are micrographs of a specimen in this orientation.

Figure 85. Apical view, that is, looking down the shank, of the syntrophium. The labral stylet is removed.

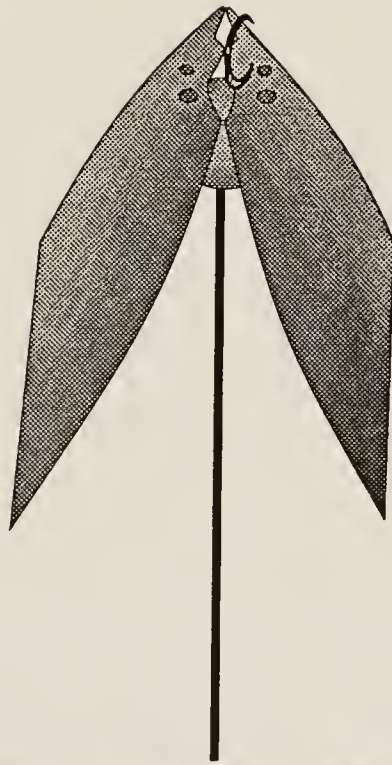


Figure 86. Closeup of oral orifice. A) The mandibles arise as extensions of the pharyngeal wall and, (B), abruptly bend to hypognathy.

Figure 87. Close up of the base, diagonal to the mouth orifice. A) The mandible narrow at attachment and, (B), its inner margin rolls over to form a crescent.

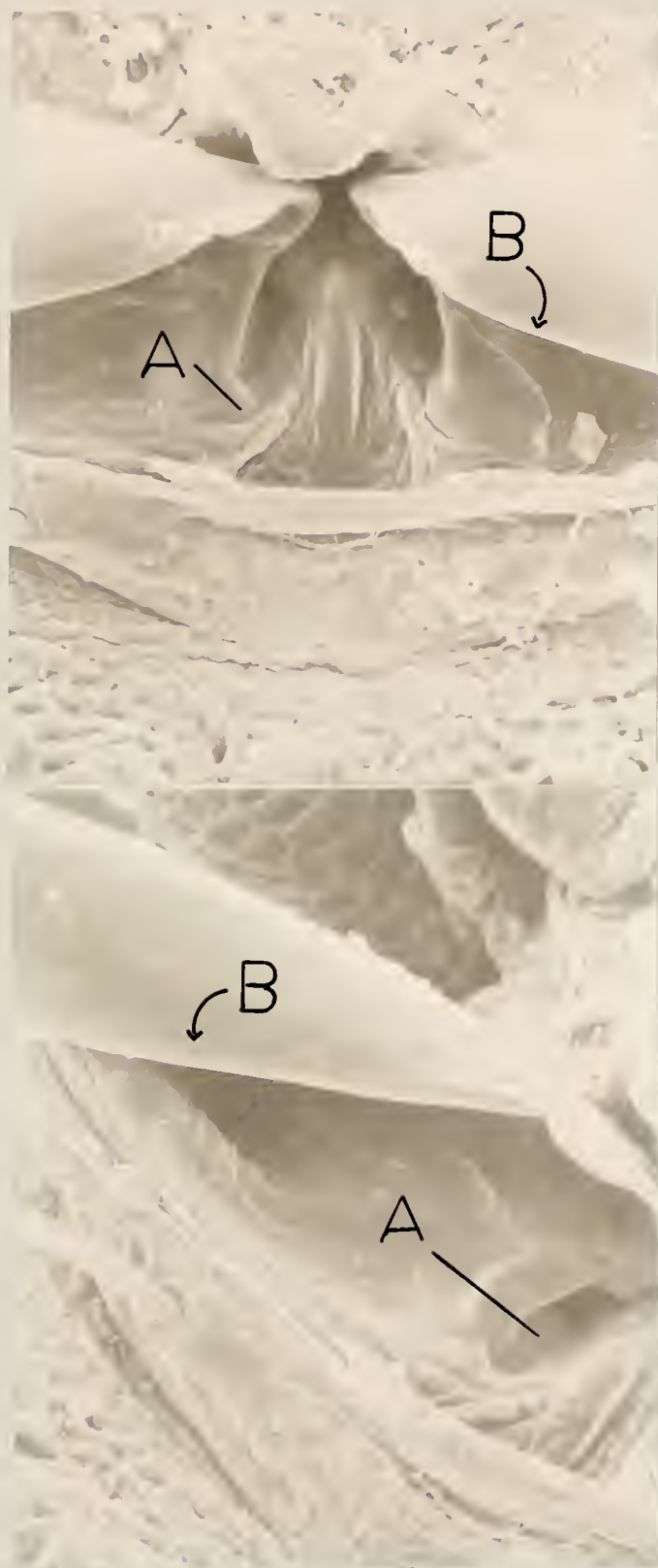


Figure 88. Anatreptic specimen of Calopteron. A) Mandible anlage.

Figure 89. Katatreptic embryo of Calopteron. A) Labrum is a transverse fold on top of the mandible. B) Central flap, perhaps referable to as the hypopharynx. A mote of foreign material is in the mouth.



Figure 90. Katatreptic embryo of Calopteron. A) Division between labro-mandibular and maxillar regions. This is the ridge shown in Figure 69.

Figure 91. Side view of Calopteron mouthparts showing three layers. A) Labrum. B) Upper mandibular lip. C) Lower mandibular lip. C) Transverse object at apex is the tip broken back by the methods of Figure 28. See Figure 92.

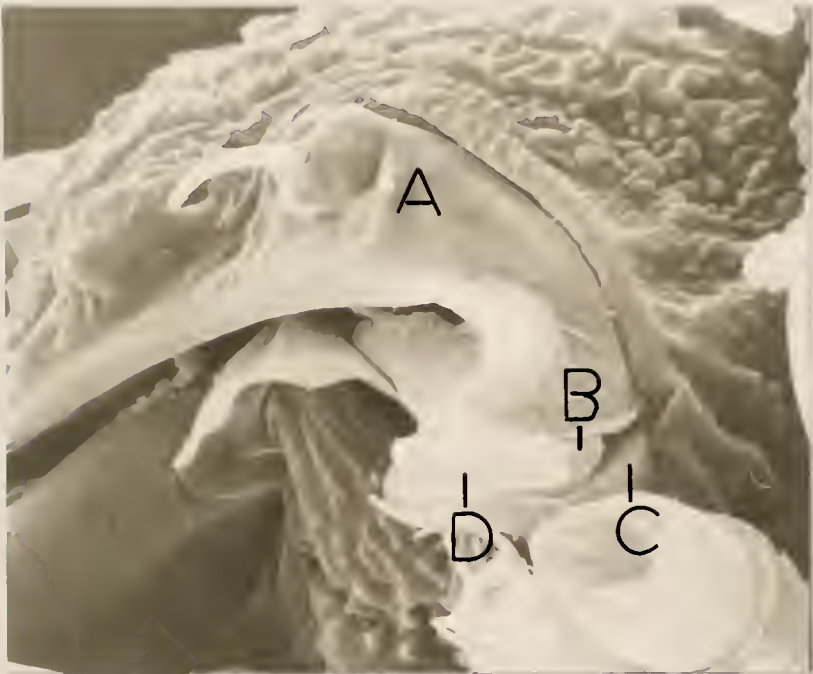


Figure 92. Close up of Figure 91. A) Labrum. B) Mandible. Both are appressed to each other as they coextend.

Figure 93. Inner ventral view of the Calopteron syntrophium in Figure 91. A) Gala is upwardly concave to receive the labro-mandibular affair.



Figure 94. Esophageal rim of a pharate larval Lycus lecontei.

Figure 95. Extreme close-up of Figure 94. Mouthpart lobes are positioned along the rim of the mouth. A) Antenna. B) Labromandibular rudiment. C) Maxillar rudiment.



Figure 96. Apolysed larva of Tenebrio with exuviae of mandible lifted off like a dental crown to reveal developing mandible within. This can be seen more clearly in the closeup in Figure 97.

Figure 97. Close up of Figure 96. A) Pharate mandible with its exuviae removed.



Figure 98. Transverse section of pharate larval mandible of Tenebrio molitor showing crenate pattern of cuticle.

Figure 99. Close-up of crenation in Figure 98.



CHAPTER 5 DISCUSSION

There can be no question that the classical designation "mandible" is, for larval Lycidae, actually a composite structure, formed from the combinatorial action of more than one anlage. By comparing embryo (Figure 89) with postembryonic instar (Figure 68), it is concluded that the labral and mandibular anlagen coextend as separate, cuticle-bound appendages, with the former on top of the latter. The two then somehow interlock before eclosion to produce a haustellate structure, complete with a food canal and continuity to the nervous system. The function of the ensheathing gala and maxillary palpifer is probably strength and support. In Lycus lecontei, at least, the tip of the gala probably serves as a food filter. This is suggested by its alveolate interior and its position between the tip of the mandible and the tip of the labrum, where the pore is located (Figure 81 through Figure 83, Figure 107).

Regarding the lampyrid syntrophium, this study reached the interpretive limits of the instruments used without definitive results. However, some good guesses can be made by going ahead with an analysis of the material available. As

will be seen, these guesses allow for a reevaluation of what it is we actually want to know.

At the moment, the central question seems to be the morphological and/or homological identity of canal and cone, i.e., what is labrum and what is mandible. Two levels of analysis, structural and cellular, can be used for determining it. The structural analysis can be addressed at this time, while the results of a cellular analysis can only be anticipated.

The Structural Level

There are two ways in which the lampyrid syntrophium might be elucidated at the structural level. One is to sample broadly among lampyrid taxa, comparing those considered primitive on other grounds with those considered advanced, as De Marzo and Nilsson did with Dytiscidae. The second argues that, because of relatedness, it should be constructed in much the same way as that of Lycidae. A comparison of the two, then, should give clues as to its actual construction.

The first alternative has to be slated as a long-term project, since most of the primitive genera occur in other countries. There is, however, no indication in the literature nor in the many specimens I've seen from North America and afar that there are significant deviations from the classical habitus of Figure 10, page 25. The De Marzo cross-section (i.e., Figure 11, inset, page 27) is the only information

available on internal structure, and I am not satisfied that it is conclusive of anything. The authors do not indicate the region of the mandible from which the section was taken. Therefore, the "fused area" they point out could be the apical or basal areas that appear cosmetically to be invaginations (Figure 42, Figure 47). Neither is continuous for the length of the mandible.

My approach to the second alternative will be to form a hypothesis on the construction of one lampyrid mesal section by superimposing a micrograph of it over a corresponding lycid micrograph (Figure 100). I shall then ask what interpretations might issue from examination of all lampyrid micrographs should the hypothesis be correct, and, finally, whether or not such interpretations are reasonable. It seems logical to use the food canal as a reference point.

The inner wall of the lycid food canal is formed from the mandible, while the outer wall is formed from the labrum. In order for this arrangement to be the case in Lampyridae, then, at the very least, cells flanking the inner half of the canal would have to be mandibular and those of the outer half labral, as in Figure 101. For the sake of argument, the other cells might be assigned identities so that they correspond directly to those of the lycid, as in Figure 102. The hypothesis that emerges to account for the difference between the lycid profile of Figure 100A and the contrived lampyrid profile of Figure 102 is that, in the latter, double layers of

cuticle (Figure 100D) are omitted. With it in mind, I shall now make morphological and developmental interpretations and, as above, ask if they are reasonable. Figure 104 summarizes the key areas examined.

The base

The lycid mandible arose as a rounded tubercle. Later, during extension, it underwent flattening and fluting into a crescentic channel such that the base is open to receive the labrum from the lateral aspect (Figure 85, Figure 104A).

The base of the lampyrid canal arose when cells pulled away from each other to form a lumen that is open to the immediate ambience, be it molting space or ooplasm (Figure 57, Figure 59, Figure 62). The function of the microvilli is not known. They may be epidermal feet (Locke and Huie 1981) that were already present, functioning to intermesh the cells before the separation, rather than new structures generated to facilitate the separation. Cuticle is laid down after the lumen is made without any indication of where cells forming the inner and outer canal wall came from.

Applied here, the hypothesis requires that lampyrid labral cells participate in some manner as in Figure 101 or Figure 102. There are no direct morphological indications that this interpretation can or cannot be so. One supporting observation is that continuity does exist between the internal cellular milieu of the presumed labrum and the trans-frontal

tissue (Figure 41, Figure 104E). This continuity is typically depicted as being achieved by connective tissue (i.e., Snodgrass 1935, 1951), but to my knowledge the origin of the cells that comprise the interior of the mandible has not been addressed (Figure 105). During exploratory dissection, I made only casual notice that tissue not only connects to the acetabulum, but also passes directly into the interior of the cone, through the opening pointed to in Figure 9B and Figure 12A. None of the cross-sections taken could verify that this latter tissue mass is actually labral.

Another supporting observation is that, immediately distal to the closure of the canal (Figure 44), the mandibular cuticle takes on a horseshoe appearance which does not embrace the lateral-most aspect. This shape is strikingly similar to the lycid mandible's embrace of all of the labrum except its lateral-most aspect, which fills the gap of the crescent (Figure 72 through Figure 80, Figure 104G). A negative observation comes from inspecting the cross-section basad of it (Figure 43). Here it can be seen that the horseshoe parts in the center so that the canal opens dorsally to the mouth. The lycid model predicts that the mandibular fluting rotate as in Figure 85, so that the canal opens interolaterally to the mouth.

Continuing, the base of the lycid mandible is narrower in diameter than the shank (Figure 87, Figure 104A,B), such that a hatchet-shape can be produced from a deftly made cross-

section. In Lampyridae, too, this configuration appears when the narrower base is included in cross-section with the broad blade (Figure 66). Such a correlation cannot be evaluated as being reasonable or unreasonable because there is no way to determine the identity of these cells and whether they migrate as in Figure 104B to form this dilation.

The shank

As discussed at the beginning of this chapter, the lycid labrum must somehow infiltrate the shank of the mandible, perhaps by growing or slotting into it during elongation of its apex (Figure 104E). In any case, this stylet maintains a position along the outer half of the mandibular shank so that the gap between its lips (Figure 104H) is filled. This dictates that the canal track be along the inner wall, which is the center of the mandible (Figure 100A, Figure 104D).

In Lampyridae, too, the line of contiguity between canal and cone follows the center of the inner wall (Figure 12, Figure 13, Figure 106), which, on other grounds referenced above, is suggested to be mandibular. Such an interpretation is reasonable only for the shank; the tracking is dorsal basally in the species compared (Figure 44), and fills most of the cone's interior distally.

During embryogenesis, lycid labral cells form their half of the lumen autonomously, before the labrum interlocks with the mandible (Figure 89, Figure 90). To be consistent with

this, lampyrid labral cells would have to make their corresponding lumenal half without influence from the other side of the mandible, as in Figure 109. Such an interpretation can only be evaluated using a cellular level analysis.

The apex

In the lycid affair, it appears that a pore is formed at the outer tip because the stylet is shorter than the mandible (Figure 107A). It is also possible that the two are capable of sliding along each other to open and close it. For Lampyridae, it was suggested in Chapter 4, page 80, that the pore is formed when the separation between canal-forming cells breaches the exterior apically.

The model being assembled would require that mandibular cells exceed the labral cells in length so as to form a tip distal to the pore (Figure 107B). The seam noted earlier (Figure 47) need not be significant. It may result from compression due to cells retreating as they lay down a tip of solid cuticle. Such an interpretation, again, cannot be evaluated given the material at hand.

Development

As discussed above, the lycid labral stylet somehow inserts itself into the mandibular flute laterally. The only indication that lampyrid labral cells also undergo such a movement is the invagination pointed to in Figure 32. A

plausible scenario of such a movement is given in Figure 108 and Figure 109. They depict labral cells flanking mandibular cells during elongation, then infiltrating into the mandible interior (Figure 109C), forming an inward crescent (Figure 109D) as previously discussed, and then pulling away from the mandibular cells (Figure 109E and F) to reveal microvilli and form the canal lumen.

In order for the apodeme model in Figure 11B to be correct, the presumptive lumen would have to be cell-filled as it migrated across the cone's diameter, since no lumen was found along this path (Figure 108E). The invagination disappears later in development suggesting that it is cast off without reconstruction (Figure 108C, D). Cross-sections of the mature syntrophium show heavy deposition of cuticle in this area, which might be interpreted as the result of synthesis from more than one field (Figure 110). Such a zone of heavy cuticle may have adaptive function as a strengthening corrugation.

Analysis of Hypothesis

All of the above are highly tenuous, yet there is substance that is compelling enough to ask some further questions. The lampyrid cell behavior modelled in Figure 109 is essentially that of the lycid affair, and I see no serious objections to the possibility that it can indeed be the case. Therefore, one timely question would be whether it matters

that intervening, double layers of cuticle be present during lycid embryogenesis.

Morphologically, it looks as if the only requirement for removing the double layers is an outward displacement of the epidermal cells constituting the basal labro-mandibular junction (Figure 111). This might be accomplished by the deletion of one protein for all we know, but would of course create a situation where labral and mandibular cells are no longer insulated from each other. The question then becomes whether or not the "cross talk" or "biochemical noise" between these exposed anlagen is detrimental to their morphogenesis. Such a question cannot be answered, except for the supposition that this situation might be comparable to the exposure that would occur with each embryonic molt. If the classical molt-growth-redeposition cycle is indeed at play, then lycid cells are moving out to form the mature syntrophium in the same way as the contrived lampyrid scheme (Figure 109), except for the cuticle-bound "embryonic instar" hiatuses. However, since nothing is known of their cytodynamics, it could also be that the components elongate while they are cuticle-bound. Since the embryonic cuticle is soft, it may not be required that cells unmarry from it before dividing and extending further.

Analysis of the Structural Level

The whole point of this teleology is to demonstrate that a fundamental question, what is mandible and what is labrum, can be far above our heads. The matter is so complex that I would be willing to argue that it is not even the right question to pose.

Traditional and contemporary comparative insect morphologists alike would pose, as their primary question, which structure is mandible and which is labrum so that their differences in shape can be evaluated in terms of the evolutionary changes that must have occurred. These structures are identified with because they seem to be readily homologizable units, discretely set off from the head and adaptively significant. Further, a concordance with respect to their size, shape and position relative to other characters is generally found across the board, from one taxon to another. The existence of this concordance, most explicit between closely related groups and least explicit among distantly related groups, has, in the past, made such a question the right question to ask.

The main obstacle to this way of thinking is that the cells forming the lampyrid syntrophium occur in the same anlage instead of two different anlagen. Whether accumulating by division of resident cells or migration of cells from

another region, this population fills the inside of the anlage and then somehow communicates amongst itself the information required to produce the canal directly, without cuticle-bound developmental intermediates. If it actually is a composite structure, then, during development, the components lose their position in serial homology and, along with it, their segmental identity. Therefore, they cannot readily be identified in terms of what is mandible and what is labrum.

For future structural level study I might suggest that the muscles servicing the acetabulum be traced to their connection on the interior of the head. As this feature has been extensively employed understanding evolution and function of arthropod mouthparts (Snodgrass 1935, 1951, Matsuda 1965), the location of this site may help in understanding the relationship between canal and cone.

If it cannot be said for certain whether construction of the lycid and lampyrid syntrophium is the same or different, then outgroup analysis cannot be performed. Further, the mandibular construction of the outgroup itself, in this case Tenebrio, cannot be reliably ascertained without first studying the possibility of anlage coalescence in its own taxonomic vicinity.

Another argument diminishing the importance of the question "what is mandible and what is labrum" and the phylogenetic principles that it might be applied to is the imposition of a common sampling artifact. The main criterion

that decided which species were used in this study was availability, rather than relatedness. One cannot be confident that observations are representative of the taxa unless a wider range of representatives is sampled.

I am very satisfied with the discovery that lycid mouthparts are composite, haustellate structures and that lampyrid mouthparts show similarities that are unclarifiable with modern techniques. Someday, analysis must be taken to the cellular level.

Anticipation of the Cellular Level

Most of the comparisons attempted above could be better addressed using a cellular level analysis were it not for the fact that cantharoids have not been reared successfully in large numbers. Cellular level techniques require hundreds of test animals for gene and protein identification and manipulation.

If these beetles were more experimentally tractable, then the cells concerned could be traced back to their origins earlier in development using the tools of Molecular Biology. Mitotic recombination markers can be inserted into these parents so that the lineage they generate can be mapped (Foe 1989, Garcia-Bellido et. al 1973). However, if beetle cells are comparable in this context to Drosophila cells, then it should be found that the clones in question do not form a pattern descriptive of any "glossary listed" structure.

Instead, some sisters might be responsible for forming the base of the antenna, others one side of the stomodaeum, and still others a chunk of the mouthpart region (Figure 112). Comparative morphologists have yet to come to terms with this inconsistency. When they do, it should be realized that the literalness of the question "what is mandible and what is labrum" has diminished severely.

Concluding Remarks

A definite distinction between labrum and mandible isn't really needed to address the original question that launched this investigation (page 2), morphogenetic relationships between Lycidae and Lampyridae evident in key character ontogeny. Focus is instead best given to the differences in the behavior of the cells involved. Examined in this light, the results do suggest one course of action, but it would be impractical even if a sufficient number of individuals were available. Labro-mandibular junctions (Figure 111) could conceivably be dissected out of enough specimens to allow for an identification of the associated proteins. These proteins could be sequenced, and their genes identified. Probes could then be made which can be used to see if the same sequences occur in Lampyridae.

As an aside, the results of this study also yielded something provocative and unexpected: the concept of two anlagen behaving according to their own programs while

confined in the same cuticular sac rather than separate sacs. It is very compelling that the hypothesized mechanism of coalescence, slippage of intervening double layers of cuticle, might be pervasive among other taxonomic groups.

The first to come to mind is Lucanidae. Their enormous mandibles may actually be formed by many cell fields that were called from various places of the head during phylogeny. Another is Adephaga, wherein hind coxae somehow fuse immovably to the abdominal base during metamorphosis. The mechanism behind this could be similar to that proposed for Lycidae (Figure 111C)- slippage of whatever anchors the epidermis into the invagination that defines the posterior coxal face.

Figure 100. Attempt to homologize (A) the lycid and (B) the lampyrid syntrophia. Trace of (B) over (A) facilitates comparison, but arrows point only to aspects of (A). a) Inner canal wall. b) canal lumen. c) Outer canal wall. d) Double layers of cuticle.

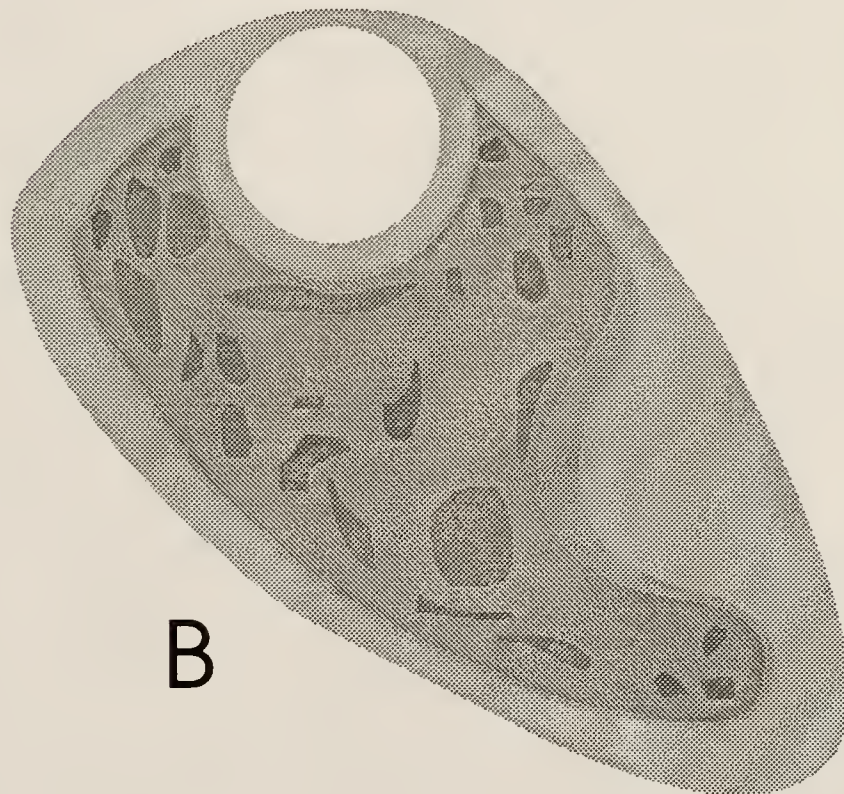
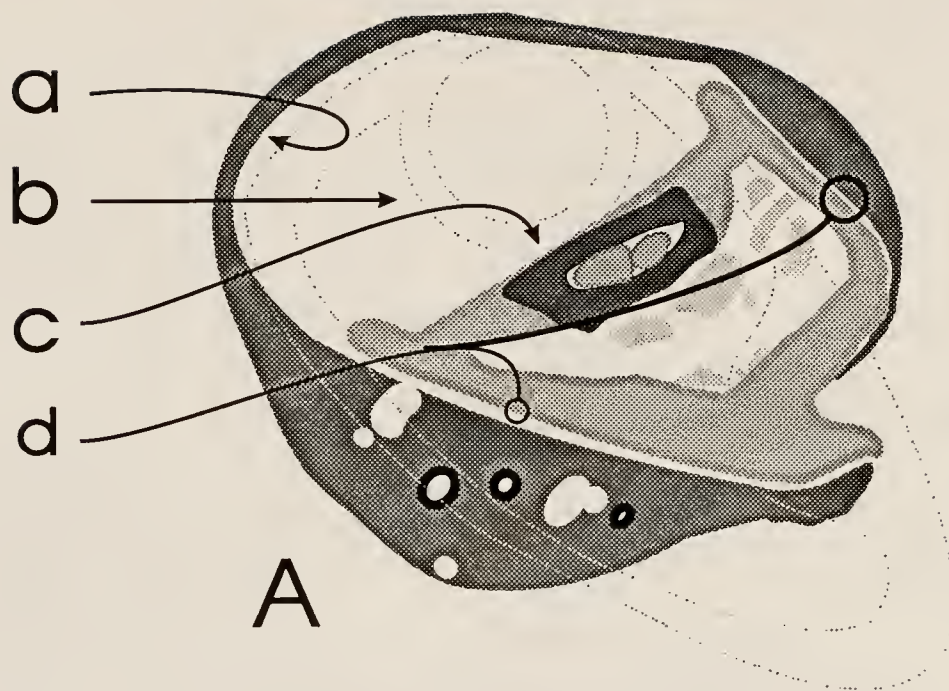


Figure 101. Minimum requirements of lampyrid syntrophial cell identity. A) These must be mandibular. B) These must be labral. C) Dotted line indicates that mandibular cells retreated after laying down cuticle in this area.

Figure 102. All cells given identities required to correspond directly to the lycid affair. A) These would be mandibular. B) These would be labral.

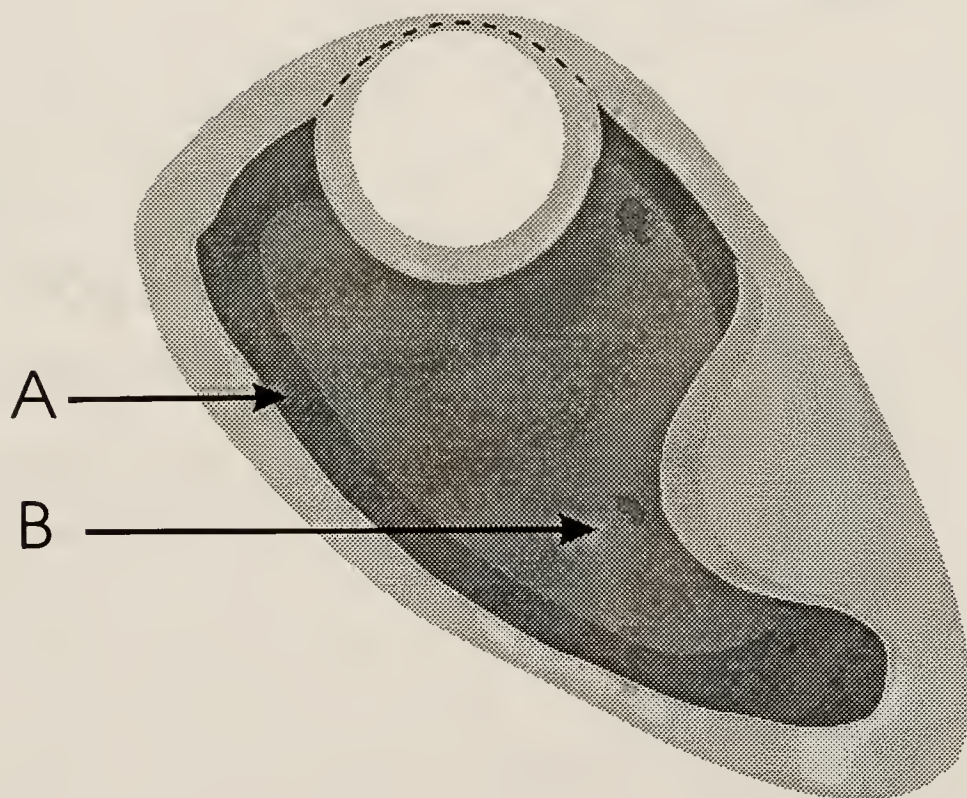
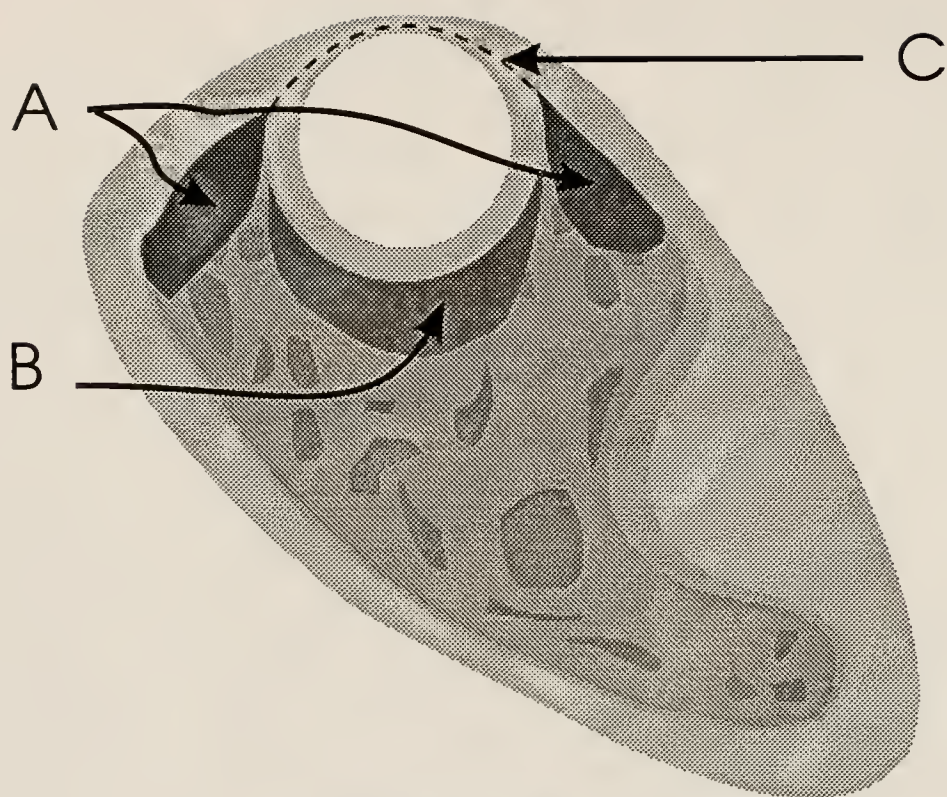


Figure 103. Close up of Pyrractomena mandible shaved as described in Methods. A) Area corresponding to the dotted line in Figure 101 and Figure 102.



Figure 104. Summary of areas used in key interpretations for both families. A) Narrow mandibular base. B) Arrows show fluting. C) Lumen. D) Center of mandible. E) Labrum is continuous with transfrontal tissue. F) Labrum undergoes fluting. G) Welt of cuticle.

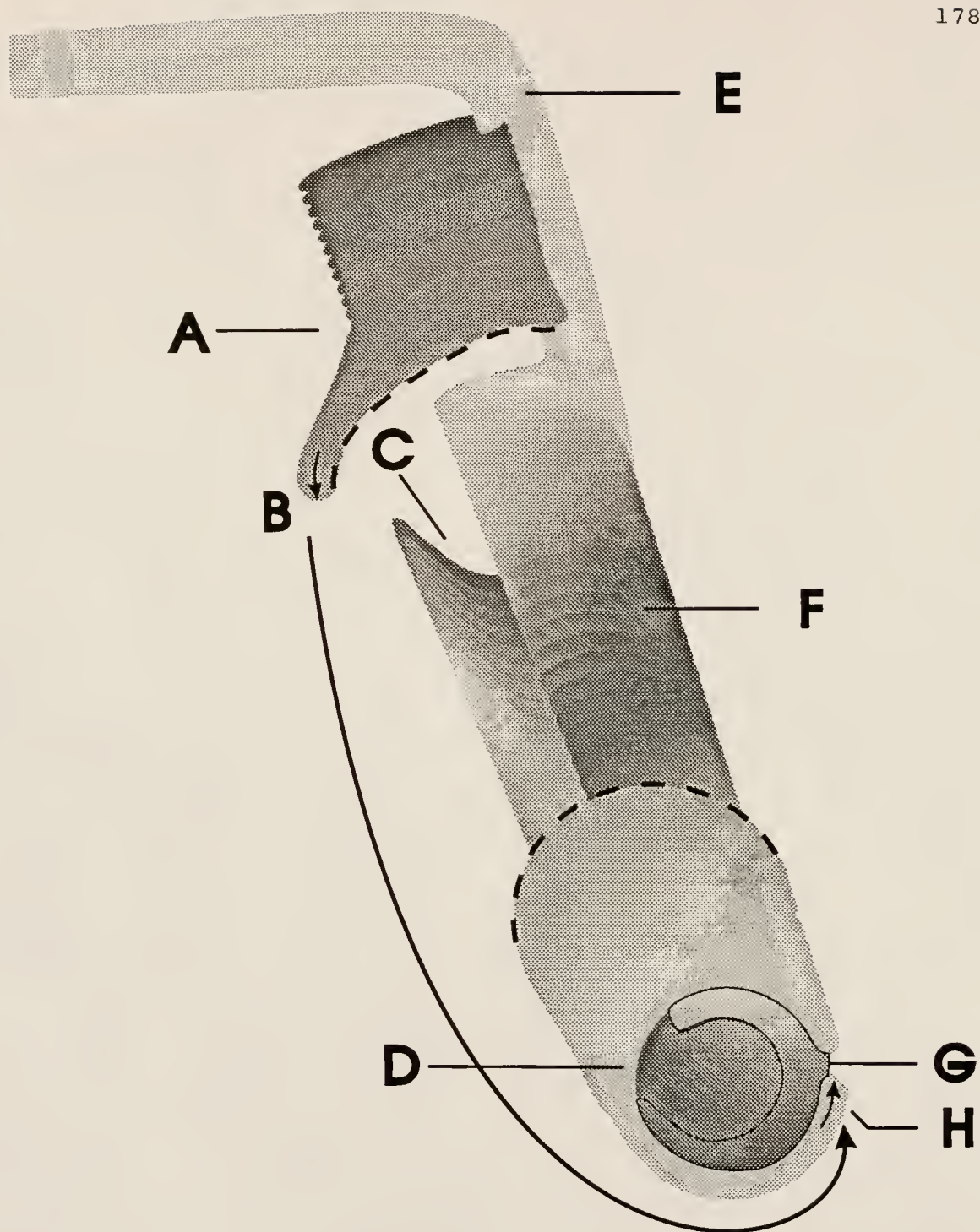


Figure 105. Typical textbook illustration of a mandible. No indications are made regarding where the cells filling the interior (cut-away) originate.

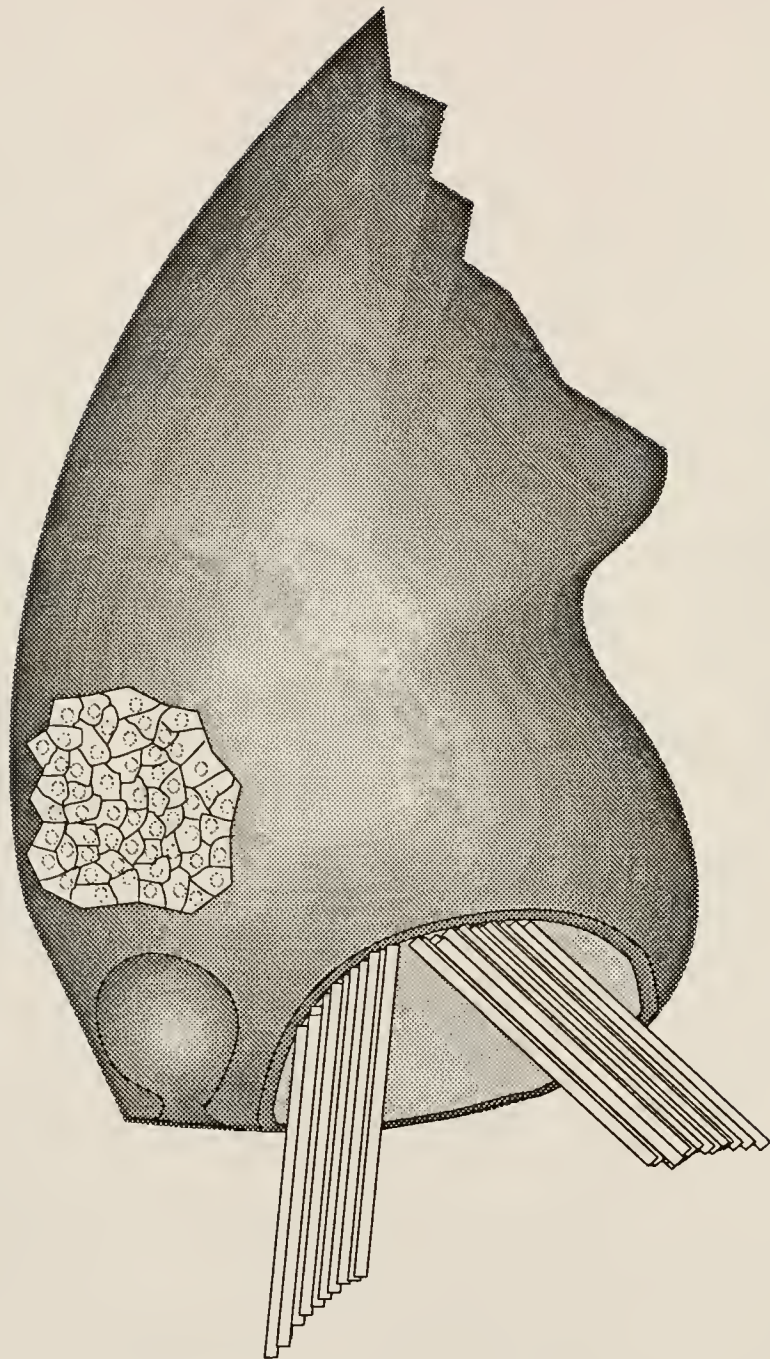


Figure 106. Tracking of the lampyrid canal.

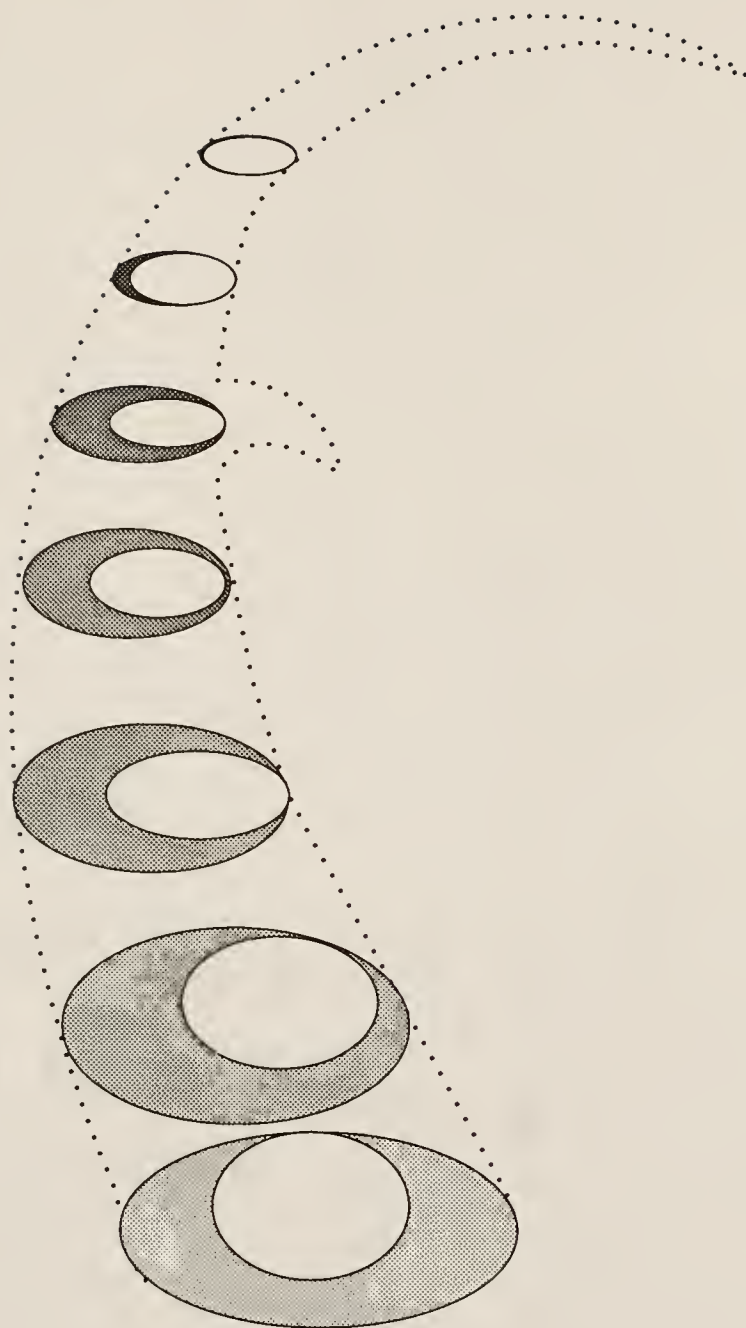


Figure 107. A) Lycidae. Labrum (a) falls short of mandible (b). (c) Alveolate gala. (d) Tip of mandible, cut for viewing path of imbibition through pore. B) Lampyridae. Pore is formed because labral field (a) is shorter than mandibular field (b).

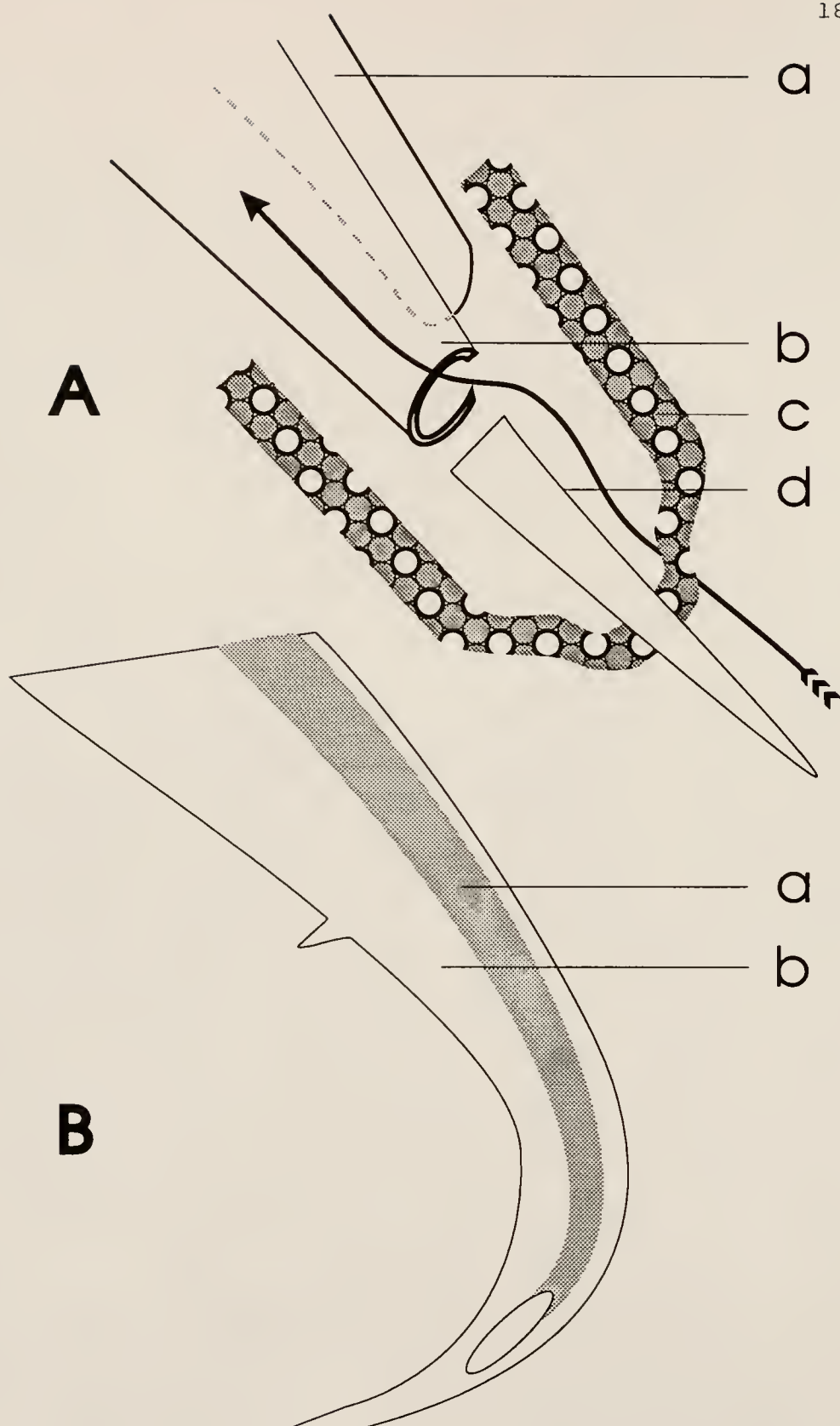


Figure 108. An interpretation of the invagination in Figure 32A. A) Mandible anlage flanked with labral cells. B) Labral cells infiltrate. C) Invagination is cast off. D) Anlage is smooth again. E) No apodeme was found.

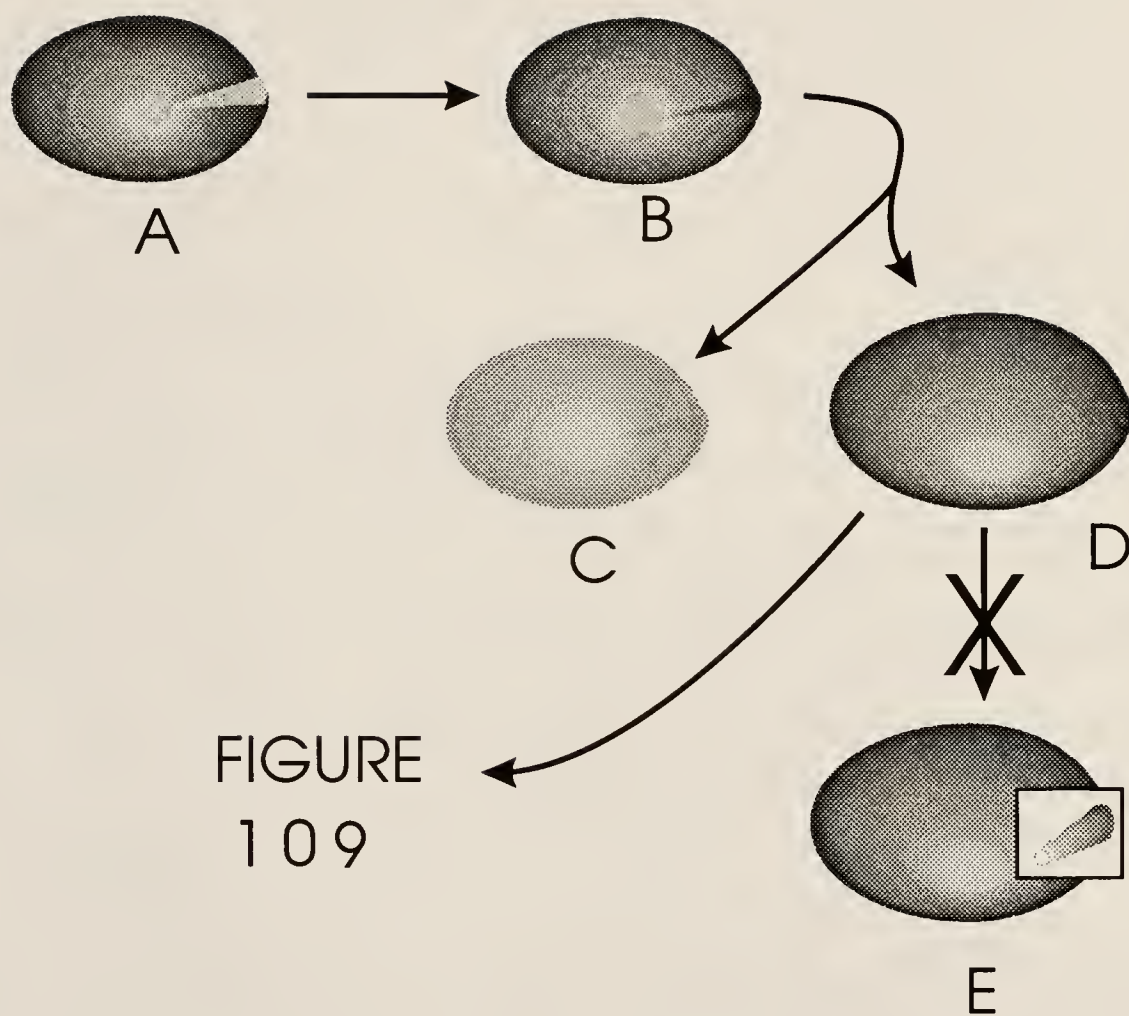
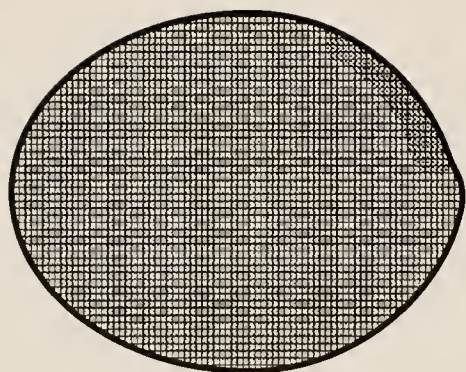
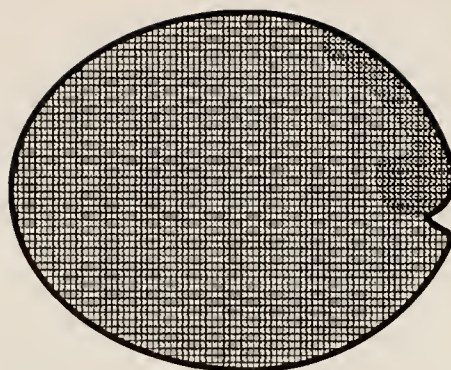


FIGURE
109

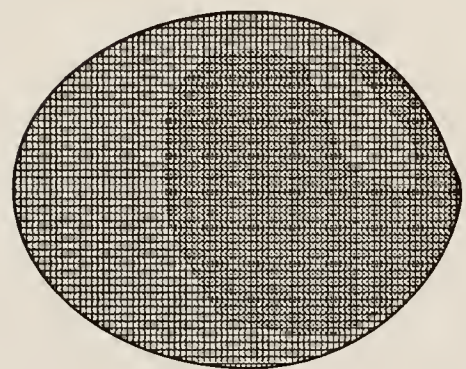
Figure 109. Cross-section of anlage in Figure 108 held static while cell fields pass through during development. A) Flank of labral cells. B) Invagination. C) Infiltration. D) Fluting. E) and F) Separation.



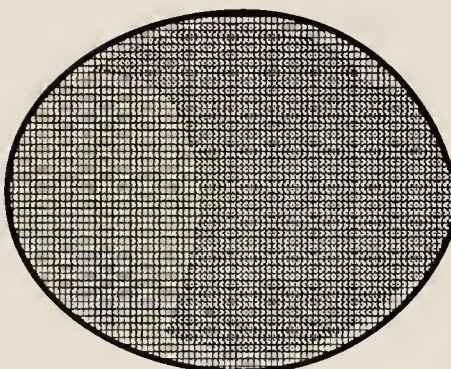
A



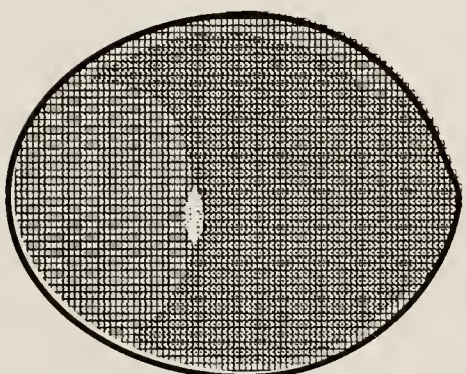
B



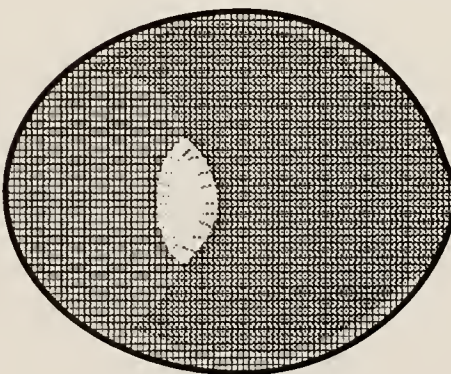
C



D



E



F

Figure 110. Mesal section of a 1st instar P. collustrans syntrophium. A, B and C show three directions from which cuticle might be deposited. A) Labrum, B) upper mandibular lip, C) lower mandibular lip.

Figure 111. Close up of the base of the lycid syntrophium in Figure 73B. A) Mandible. B) Labrum. C) Labromandibular junction. The junction need only be displaced outward, in the direction of the arrow, for the two anlagen to be made one.

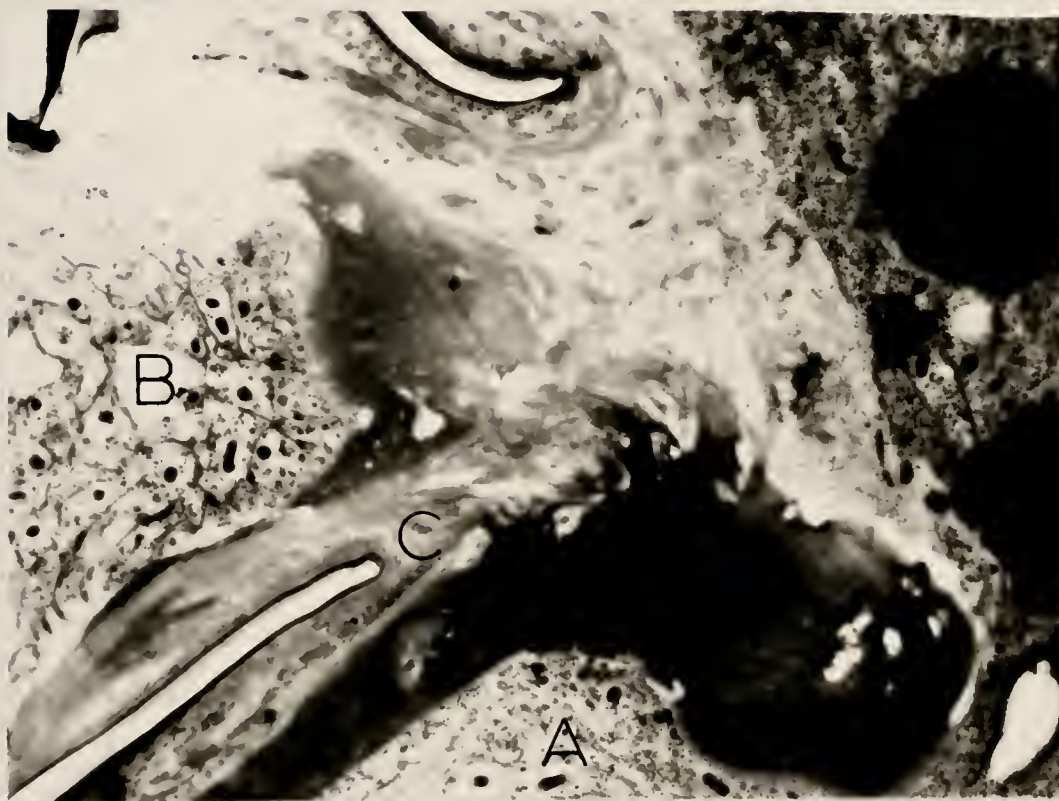
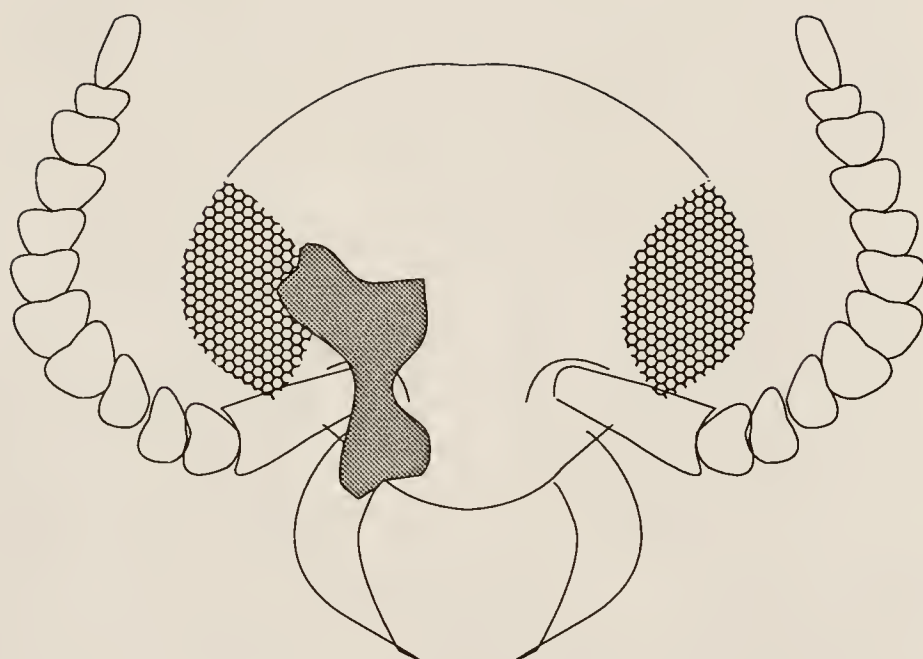


Figure 112. Generalized beetle head capsule overlaid with a compartmentation pattern that might be expected from what we know of Drosophila cell behavior.



APPENDIX
A THOUGHT EXPERIMENT IN HORIZONTAL SALTATION

Horizontal saltation can be defined as a morphogenetic event that produces a morph of a different taxonomic group from the morph developmentally or reproductively prior to it. This definition would encompass a change between instars as well as between generations.

Before commencing the experiment, certain initial conditions must be met: The more pedestrian alternatives, parasitism and saprophagy must be given as much consideration as possible. After the information available on the likelihood that one of these possibilities might be in operation has been exhausted, I shall begin the experiment by assuming, for the sake of argument, that the siblings described in the Introduction actually are products of the bearer's genome. The experiment is carried out with no intention of conferring a sense of advocacy to the reader. I consider it to be merely a thought-provoking exercise.

Parasitism and Saprophagy

The siblings cannot be associated with any taxon lower than family because a classification scheme for larval lampyrids has not yet been developed. However, color and

chaetotaxy, but not body width, of the siblings is consistent with what is known of the subfamily Photurinae (Cicero 1988).

The bearer was collected in north-central Tucson, Arizona, an area with a long history of avid entomological study. The only ecologically sympatric lampyrids are Pleotomus nigripennis LeConte, Pyropyga nigricans Say and Microphotus dilatatus LeConte. All three are assigned to the subfamily Lampyrinae, and M. dilatatus was the last to be discovered, about 80 years ago (Fall 1912). All instars of the first, late instars of the latter two, and oviposition of M. dilatatus are known. Their structure and behavior at the time of collection cannot be associated with the phenomenon at hand.

Two glowworms, Distremocephalus opaculus (Horn) and Cenophengus ciceroi Wittmer, (Phengodidae), and at least two long-lipped beetles (Telegeusidae) should be given consideration too, because they are ecologically sympatric, assigned to Cantharoidea, and their larvae, females and behavior are unknown. Males of these phengodids are ca. 5mm long and anomalous. The general profile of Phengodidae is based on Zarhipis and Phengodes, which have large, larviform females and millipede-preying larvae (Tiemann 1967; unpubl. observations). Telegeusidae are a small family, the males of which are 5mm long or less, nonluminescent, and possess flabellate palps. Their females may perhaps be large and larviform too. At present there are no morphological grounds for reconsidering the males of Phengodidae and Telegeusidae to

be lampyrids. Nor is there reason for suspecting that any of their larval instars are lycid or generalist parasites or possess lampyrid morphology. Cantharidae are ubiquitous, but their larvae are profiled as predators in detritus (Bøving and Craighead 1953; Peterson 1982).

About 14 other lampyrid species are regionally sympatric and, similarly, suspicion of a parasitic or saprophytic habit cannot be mustered given what is known of their larvae and what is expected as members of the family. These are Bicellonycha wickershamorum Cicero, Ellychnia bivulneris Green, E. n. sp., Pleotomodes pulsator (Cicero), 4 species of Microphotus (Green 1959), Paraphausis exima Green, Photinus knulli Green, P. pyralis (L.), Pyropyga minuta LeConte and P. modesta Green.

The world over, only aquatic (Blair 1927) and myrmecophilous (Cros 1925; J.E. Lloyd pers. comm. [Pleotomodes]) behaviors are the exception to essentially stereotypic (Sivinski 1981) habits of larval Lampyridae. However, this profile is based on mid-instar larvae, and of a very few species. Oviposition habits and early instars are more pertinent to the discussion, and these are unknown for virtually all of Cantharoidea. The idea that an adultless lampyrid may exist by subsisting as a parasite and reproducing paedogenetically may sound ridiculous, but objectivity requires mention for the simple reason that it is just as imaginative as the other propositions are. Micromalthus

debilis Leconte (Micromalthidae) can be cited as a precedent. The sexual cycle of this species, adult female mating with adult male and laying eggs, seems to play a minor role in reproduction as only 1 or 2 eggs are laid. Scott (1937), on failing to rear them, suggested that it might even be undergoing obsolescence. Conceivably, the larvae could eventually assume full role in perpetuation of the species. This beetle is covered in greater detail later.

It should also be noted that the presence of a cord linking two groups of siblings to host or mother is not consistent with known behavior of parasitic and parasitoid insects. If these two groups are truly a reproductive feat of the bearer, then they would correspond to paired ovaries as is seen in Micromalthus (Scott 1941:429). The cord might then be trophic or respiratory. It is probably not a remnant of the bearer's tracheal system as it issues from within the anal end of each sibling. The tracheal system was probably masticated as there were no remnants in the gleaned interior.

The Experiment

Given that the siblings are a product of the bearer's genome, the birthing can be called paedogenesis. As a start, I shall generate ideas from the standpoint that the jump might be a normal event in lycid life cycles that has gone unnoticed. Then I shall discuss the considerations that come to mind should the jump be a freak accident.

Paedogenesis will be discussed with special reference to hypermetamorphosis, since lampyrid characters may have gone unnoticed as a normal aspect of early lycid life-cycles. Hypermetamorphosis refers to different larval instars in the life-cycle of the same individual. Classical hypermetamorphosis is applied to such insects as blister beetles and Micromalthus wherein 1st instars, called "triungulin", are adapted for dispersal with their long legs and rapid running ability. Subsequent larval instars lose the legs to become sedentary feeders. Legs are then somehow reinstalled on the adult during pharate pupation.

Horizontal gene flow (Dawkins 1982, Doolittle et. al 1990, Shatters and Kahn 1989) evokes very little discussion and will not be included in this experiment.

The Birthing as a Normal Event

Paedogenesis

In paedogenesis, eggs develop in the larva and are activated parthenogenetically. Embryos develop into larvae which consume the mother's tissues and are born either through the vaginal tract if one is present, or a rupture in the cuticle. Paedogenesis is recorded in midges (Chironomidae, Tanytarsus, Johannsen 1911), flower flies (Syrphidae, Eristalis, Ibrahim and Gad 1975), blow flies (Calliphoridae, Calliphora, Parker 1922), gall midges (Cecidomyiidae, Mycophila, Wyatt 1967, Sanui and Yukawa 1986, Aprionus, Prasad

1968), Micromalthus debilis (Micromalthidae, Scott 1937), springtails (Wootton 1984), termites, (William Nutting, pers. comm.), aphids (Wood and Starks 1975) and bedbugs (Hagan 1931). Hemipupal paedogenesis is known from several cecidomyiid genera (Wyatt loc. cit.). Lastly, the pupa of a bagworm moth (Psychidae) was collected into preservative with hundreds of psychid larvae inside her and issuing forth through a rupture (Florida State Collection of Arthropods, Gainesville, labeled "Oiketicus abbotii Grote, det. J.Heppner" and "Miami, Florida, M.Thurman, coll., 3-xi-83, on Terminalia catappa)."

There is no reason to think that Lycidae or any other family is immune to the experience. Many parthenogenetic egg activation mechanisms have been published (i.e., Tyler 1941; Went 1982), but nothing known of Lycidae suggests which might be involved here.

A survey of the world literature on the larvae of these families is in order, as something relevant to any of the above topics may have been missed in collation of the primary literature.

Larval Constitution of Lampyridae and Lycidae

In addition to what might be called "Lampyridae proper", there occurs a suite of genera whose adults are morphologically intermediate between Lampyridae and Phengodidae (Cicero 1988, Crowson 1972). These will not be

considered here. The remaining ca. 70 genera are sufficient to cover the world distribution of the family.

Twenty-two of the 70 have at least one reliably associated larva. Quality pictures and descriptions date back as far as the early 1900s (i.e., Vogel 1915), and indicate that no matter where in the world they originate, the following can be considered consensus characters: a head capsule as in Figure 3, left, light organs in dorsally fenestrated, ventrally convex, penultimate abdominal pleurites (Figure 5, Figure 6), and an anal proleg (pygopod) composed of barbed, retractile vesicles (Figure 113). Other characters, such as leg length, chaetotaxy, and sclerite configuration help to solidify the larval definition of the family. Using orthodox thinking, another 12 of the 70 are, by their close association with one or the other of the 22, expected to have larvae with these characters also. For example, the adults of Photinoides, Macrolampis, and Diphotus are so closely related to Photinus to expect that their larvae are photinoid also. Further, unidentified larvae possess these characters also, which strengthens the contention that lampyrid larvae (proper) are consistent in structure around the world.

Lycid life cycles, on the other hand, are considerably less well known. Over the years, only 16 of the ca. 150 genera have accumulated one or more larval descriptions. As with Lampyridae, consensus characters extracted from them and certainly from miscellaneous larvae unassociated with their

adults, have solidified into a larval definition of the family. These are the cleft mandibles (as interpreted at that time, Crowson 1972) and the absence of a frontal suture. Other features can be taken as diagnostic on inspection of published figures. These include a head capsule as in Figure 114, short legs, distinctive sclerite configuration and an undivided, tubular, non-barbed anal proleg (Figure 115).

Only one questionable report was located. C.S. Papp (1951) describes, figures, and assigns to the lycid genus Calochromus a Javan larva of lampyrid morphology. Since it is not positively associated with an adult, and since Calochromus larvae had already been described as having typical lycid morphology (Gardner 1946), it seems likely that this is a misidentification. Consistent with the definition of Lycidae, first instars reared from eggs of L. sanguineus, late instars reared through to adults of L. sanguineus, and the bearer itself possess mouthparts so modified, and also possess the other features as well. The siblings bear the lampyrid characters in their totality, and lack the lycid characters in their totality. L. sanguineus pre- and postkatatreptic embryos too, show no transtaxonomic characters.

The Birthing as a Freak Event

Assuming these records accurately reflect the actual constitution of the families, then there is no immediate reason to further consider the possibility that lampyrid

characters have gone unnoticed as a normal aspect of early lycid life-cycles or vice-versa. The discussion returns to paedogenesis.

Three ways can be imagined in which paedogenesis could be a freak gateway to another taxon. 1) Early toggling to another developmental pathway that involves a different set of genes than is normally used. 2) Extension of the normal developmental pathway by prolongation of whatever mechanism controls the duration of embryogenesis (Sivinski, personal communication) 3) Gross chromosomal change.

Curiously enough, the criteria involved here, paedogenesis, complex developmental pathways and bizarre chromosome behavior, occur in Micromalthus and Cecidomyiidae. Therefore, these practitioners should be reviewed also.

Review of Other Practitioners

Developmental

The life cycles of Micromalthus and cecidomyiids are basically similar in structure (Camenzind 1977, Scott 1937). It seems clear that in both, the default reproductive cycle has shifted to a unisexual one, wherein larvae develop into paedogenetic mothers and give birth to daughters but not sons. The cycle repeats for as long as environmental conditions are favorable. During this unisexual cycle, Micromalthus is hypermetamorphic- first and possibly second instars are triungulin while subsequent instars, perhaps 2, are legless wood-borers. Body shape in both is orthosomatic as opposed to

the crescent-shaped (cyphosomatic) body form of scarab and weevil grubs. For the cecidomyiids, hypermetamorphosis is not reported within this cycle, although a yellow form has been described for Miastor in contrast to the normal white form.

When stressed with desiccation, starvation, etc., a change in reproductive attitude is initiated such that sisters are capable of escaping to the sexual cycle (Went 1975; Harris 1925). Some develop directly into exarate pupae, then adult females. Others give birth to daughters which, facultatively or not, give birth to sons. Sons develop into exarate pupae, then adult males.

The adult stage of Micromalthus is thought to be protected from obsolescence by its selective advantage in dispersal. However, considering the acknowledged morphological correlation to triungulin of other families, the possibility of phoresy should be considered.

Adult reproductive capacity in Heteropeza can be parthenogenetic or bisexual, in Mycophila reportedly bisexual only, and in Miastor uncertain. Adult females of all four produce daughters only, which then reinitiate the unisexual cycle.

Generation turnover is extremely protracted. For example, in Heteropeza, the eggs of the next generation form in one-day-old sisters. Because of this, the physiological portal of exit from the asexual cycle is not readily identifiable. Said another way, it is not clear whether induction involves 1) a

change of developmental pathway in mothers, as yet born or unborn, such that their daughters escape the asexual cycle by proceeding to pupa or by bearing sons, 2) a change of developmental pathway in sisters, as yet born or unborn, such that they escape the asexual cycle in one of the same two ways, or 3) a change in behavior of chromosomes across a change in generation. All three and perhaps other mechanisms are possible.

In Micromalthus, newborn daughters escaping the asexual cycle, either by developing directly into pupae, then adult females, or by bearing sons, are triungulin too. In fact, they are indistinguishable from those of the asexual cycle until after they lose their legs, when the eggs within them have developed to a diagnostic age. Egg shape, as seen through the thin larval cuticle, is characteristic for all reproductive types. The male-bearing mother is oviparous. Two or three eggs are produced but only one is shed, which adheres to her outside cuticle. On hatching, the male larva, legless and crescent-shaped in contrast to the triungulin, inserts his head into her genital aperture and consumes her. If he is removed before harming her, she will generate another batch of eggs which, surprisingly, will hatch into triungulin.

In Heteropeza, newborn daughters and sons escaping the sexual cycle differ from sisters of the asexual cycle in possessing imaginal discs (Harris 1923). This is the only character diagnostic of the sexual cycle that is specifically

reported to occur at birth. Other characters are described which distinguish the sexual from asexual cycle but the timing of their appearance is not so tabulated. The idea here is that characters most likely to be associated with meiosis are those that appear at birth rather than during the course of development. Meiosis, in turn, is a primary focus in evaluation of the lampyrid birthing. In Heteropeza and Mycophila at least, if a sister destined to adulthood is relieved of stress, she can revert back to the autoreproductive cycle in spite of the presence of these characters.

Genetic

Early embryogenesis has been studied extensively in the asexual cycle (that is, sister embryos inside their paedogenetic mothers) of Heteropeza pygmea (germ-line $2N=55$, 58, 66 or 77 depending on the race, Panelius 1968), Miastor sp. (germ-line $2N=38$ to 40 depending on the individual, Nicklas 1959) and Mycophila speyeri (germ-line $2N=29$, Nicklas 1960), but not in Micromalthus (germ-line $2N=20$, Scott 1936).

Embryos develop by apomictic parthenogenesis wherein the first maturation division occurs in regular fashion but the second maturation division is omitted. One of the two diploid nuclei degenerates, the other is the primary cleavage nucleus. In Heteropeza and Miastor, the first and second cleavages occur normally, and at completion the four resultant nuclei are antero-posteriorly spaced out so that one is closest to

the nutritive chamber while another is closest to the pole-plasm. During anaphase of the third cleavage, a certain number of chromosomes of the anterior three fails to egress, and with completion of telophase, are eliminated into the ooplasm. The posterior nucleus divides without chromosome elimination and one of its daughters enters the pole-plasm to become the future germ cell nucleus. The other daughter remains somatic; it, and the other six, undergo further chromosome elimination as early cleavages continue such that all somatic cells have a net $N=10$ in Heteropeza and $N=8$ in Miastor. In Mycophila, the first 3 cleavages proceed normally, the posterior most of the 8 enters the pole plasm and the other 7 undergo elimination of 23 chromosomes leaving somatic cells with a net $N=6$. In all these practitioners, the germ-line divides according to its own program and without elimination. Therefore, the germ of subsequent generations of a perpetuated asexual cycle always contains the original ploidy while the soma is constructed by only a fraction of them.

Bizarre chromosome behavior also occurs during early embryogenesis of interpolated sons, in the adult female, and in spermatogenesis (Camenzind and Fux 1977).

It is not known if the same chromosomes are eliminated at the turnover of each new generation. Nor is there any indication that changes in morphology and reproductive attitude inherent in the life cycles is the consequence of this elimination phenomenon. That is to say, there is no

indication that the destiny of any given sister depends on which chromosomes are spared and which are eliminated. The eliminated chromosomes are interpreted as polyploid with some indication of aneuploidy, but the possibility that they are associated with the definition of other taxa has not been considered.

Paedogenesis and bizarre chromosome behavior need not necessarily be the consequence of each other, for non-paedogenetic cecidomyiids show chromosome elimination too (Geyer-Duszynska 1958; Bantock 1970).

Analysis of Review

Developmental

In cecidomyiids and Micromalthus, there are indications that change in developmental timing is involved in producing the various morphs. D.F. Went has recognized that a heterochronic shift occurs in early development of Heteropeza: cytological aspects characteristic of embryogenesis commence during, rather than on termination of, oogenesis (Went 1979). This could certainly be pursued as the basis for determining if first instars of the asexual cycle are different (i.e., lack wing discs) from first instars of the sexual cycle because they are developmentally more larval.

Regarding Micromalthus, it might be that the legs of the triungulin are formed by the same mechanisms that confer legs to the pupa. Further, the crescent shape of the male Micromalthus larval body, as well as that of scarabs and

weevils, might be an embryonic cross-section of the orthosomatic body-type. Orthosomatic larvae are also constrained to a crescent shape while still in the egg, and the mechanism for this constraint is not known. Should the mechanism be a slower proliferation of cells along the ventral midline than along the dorsal midline, then it can be argued that crescent-shaped larvae result from quitting the embryo sooner than straight-shaped larvae. Such ideas have, to my knowledge, never been explored.

But whether developmentally based or otherwise, current thought holds no reason to suggest that the hypermetamorphic instars of cecidomyiids and Micromalthus are or were associated with other taxa. The fly larvae are relatively nondescript insofar as they lack sclerites and other somatic features, including a definitive head capsule, which might otherwise be recognized as transtaxonomic.

Just as significant as change in developmental morphology, there also occurs change in reproductive attitude. Both might conceivably apply to what happened with the bearer, but the review fails to shed light on their mechanisms.

Genetic

One question of direct bearing is whether other families of Diptera are based on the original ploidy of these cecidomyiids. There is no knowing what these flies might metamorphose into should certain or all chromosomes be spared. This question would come from studies that involve blocking

elimination in somatic nuclei rather than those that involve inducing it in pole nuclei.

Relevance of the Review to the Birthing

The review indicates that a great deal of plasticity occurs in the development and cytogenetics of these insects, but I see only one aspect that can be related to the issue at hand. A mechanism does exist for phenotypic segregation-genotypic conservation of subsets of the genome, a model that plausibly fills requirements to invoke horizontal saltation.

The only genetical research done for Lycidae and Lampyridae is karyotypy. Lycids thus far examined are $15+X$ (2 species), $15+X+s$ (2 species), and $16+X$ (2 species). For Lampyridae, $7+X$ (1 species), $8+X$ (1 species), $9+X$ (20 species), $9+X+s$ (1 species) and $9+X+0,1,2,3$ or $4s$ (1 species) have been documented. Most, if not all, of these data are from spermatogenesis (Wasserman and Ehrman 1986).

Given that lycid counts are roughly twice those of lampyrids, the hypothesis first comes to mind that a certain subset (7 to 9 chromosomes) of the $15N+X$ lycid genome is actually the lampyrid genome which can be instated by an elimination process. If such is the case, a Dawkinsian thinker might then follow with the idea that genes are eluding predators and parasites by escaping to another clade. However, there is no indication from chromosome size-lineups that a lampyrid genome could be a subset of a lycid genome (Virkki 1963).

Given that the siblings actually are manifestations of the bearer's genome, then the mutation had holosomatic rather than mosaic effects. Mosaics are sectorial disturbances and can be structural, as reported in robber flies or regulatory, well-known of Drosophila and hind-limbed whales (Cooper 1990; Lewis 1982). In the latter cases, abnormality does not persist clear through to the end of the structural gene hierarchy, instead, the result of the regulatory disorder, be it a wing or leg, is built using contemporary tissues. For them, the term "atavism" is used in the broad sense because it is reasonable to interpret their abnormalities as reinstatements of ancestral developmental pathways. The opportunity now comes to explore the use of the term "atavism" in the literal sense.

Literal atavism

Reappearance of a species that actually existed in the past is a novel concept. Orthodox reasoning would have the minimum criteria required to interpret the bearer as a literal atavist be that 1) Lycidae are an outgroup of Lampyridae, 2) the stem species have the suite of lampyrid characters used to identify the siblings as such, and 3) the reversion is a jump to somewhere within a time period that starts with this stem and proceeds thereafter until the suite is no longer present on this parental stock.

Regarding the first requirement, if Lampyridae were an outgroup of Lycidae, then the birthing would be convergence, not atavism. Regarding the second, if the stem species of

Lycidae did not yet have the full set of contemporary lampyrid characters seen on the siblings, then the birthing would be convergence also. It would be fruitless to belabor these points any further. The third requirement provides the greatest insight into the problem.

The third requirement allows for the siblings to belong to any of an inestimable number of ancestral species within this time period. Chance or predilection might have determined which one L. sanguineus will give birth to. Which one depends upon whether micro- or macroevolution was involved in forming Lycidae. When thinking within the confines of microevolution it would be chance, and the placement of the lycid stem relative to the conception of the suite would be critical since this school of thought confers no distinction to any one species over all the others along the lineage with regard to the likelihood of rematerializing in the future. The closer the lycid stem is to conception of the suite, the higher the improbability that the saltation actually land between them. Should Lycidae have been conceived from Lampyridae by a one-step Goldschmidtian feat without leaving intermediates, perhaps preceded therebefore and followed thereafter by microevolutionary change, then the consideration of what species along the lineage is likely to reappear in the future would focus on the mechanism of the feat, and envision it as reversible. The birthing of a lampyrid rather than something else, either earlier (i.e., some protolampyrid) or later

(i.e., a lycid-lampyrid intermediate or a true lycid), as would be likely in a microevolutionary anagenesis, certainly suggests that such reversal has indeed occurred. The suggestion, of course, is viable as long as it remains in the same theoretical plane established for this discussion.

The tendency to invoke atavism is natural and seductive, due to the assumption that the most likely genetic access route to another taxon is by way of a stem, unique and common to both. For the lycid this may not be correct since the characters generated are diagnostic of another, extant, clade. It is because of this preoccupation that horizontal saltation rather than retrovertical saltation (atavism) must be considered. If the siblings would have metamorphosed to a lampyrid species known from one place or another within the likely historical distribution of L. sanguineus, currently known as Arizona east to Texas and south to Sonora, Mexico, then it follows that the saltation has occurred at least once before. Such a line of thought is provocative as it leads to such ideas as a biphyletic species or an alternation of species. A more conservative idea is that the paedogenetic mother was a potential stem individual to a new lampyrid taxon. But the point is that with such questions, it is now conceivable that members of Lycidae past and present might, by way of this transfamilial mode of reproduction, be ancestors to at least some of a polyphyletic Lampyridae. Many lampyrids are behaviorly no more complex than lycids. Similarly, without

an understanding of the mechanism of the jump, for all we know, Lampyridae may be, or may have been, capable of generating a polyphyletic Lycidae too.

In other words, at least one reasonable alternative to atavism is horizontal saltation, wherein two morphofamilies have been crosslinked in parallel through the ages, escaping predators and parasites by switching between clades, instead of maintaining evolutionary separateness during divergence as has previously always been thought. In taxonomic terms at least, it is justifiable to refer to such a jump as horizontal. As a final consideration, the adult fauna should be reviewed for any indications that horizontal saltation was involved in their biogeography.

Adult Constitution of Lampyridae and Lycidae

Lampyridae and Lycidae are cosmopolitan (McDermott 1966; Kleine 1933). Lampyrids hold about 1900 described species in 92 genera and the lycids about 3500 described species in 150 genera (Lawrence 1991). Their placement in Cantharoidea has remained stable, but there has been disagreement on a closer estimation of their relatedness. Historically, and in chronological order, lycids have been considered a tribe (LeConte 1861), subfamily (LeConte 1881), and familial outgroup (Sharp and Muir 1912; Pototskaya 1983) of lampyrids, and have also been placed near the stem to the superfamily (Crowson 1972). Such differences of opinion are not surprising

considering the incidence of neoteny (Cicero 1988), luminescence, and meiotic pre- and post-reduction (Virkki 1978), three formidable, major criteria required in their reconciliation. Crowson (1972) considers that a major impediment in the reconciliation of the superfamily has been that life cycles of such taxa as Omethidae remain unknown.

These rough estimations of relatedness are of little consequence to this discussion because of its initial conditions. Given that the birthing is authentic, it falsifies basic tenets of the schools of thought from which they were drawn. Still, in attempting to estimate degree of relatedness, these authors have also sorted the species phenetically. This grouping certainly helps in reappraising the literature. In the New World at least, there are no known species of questionable placement between these two families. It cannot be said that such a systematic gap exists between the two families in the Old World, however, because of the conceivably transitional Homaligus and Pristolytus, that have not yet undergone modern analysis. A conspicuous transitional fauna consisting of many genera does occur between Lampyridae and Phengodidae in both Old and New Worlds (loc. cit.). No transitional fauna is known between Lycidae and any other family.

Biogeographical studies have shown another systematic pattern- distributional gaps exist within these two families. In fact, it is apparently a peculiarity of Cantharoidea in

general that very few or no genera of any given family are common to different major land masses (McDermott 1960, 1964; Crowson 1955). Systematic gaps are what one would expect if Lampyridae and Lycidae were actually phylokaryotypic versions of each other.

Concluding Remarks

No doubt this discussion can be continued ad infinitum. I have taken it this far because I think it is important to seize advantage of opportunities to contradict established doctrines. The old saying that "like produces like", still has meaning; concepts such as evolutionary separateness and cladistics would not necessarily be threatened by this discovery if it is authenticated. Authentication would simply prove that at least one gene system exists which these two doctrines cannot accommodate. In the final analysis, the problem may only be terminological. I say this because, as it stands now, the putatively authentic birthing is understandable when thinking at the level of the gene but not at the level of the species. Such a dilemma has already been overcome for the conceptual breakthroughs that occurred in population biology, adaptation, natural selection, etc. (Fisher 1958, Williams 1966), and it is conceivable that the birthing could be a seed for the same sort of revolution. Molecular biology has produced another seed, one which is already germinating and may soon revolutionize our conception

of mutation as the agency of speciation. The intense studies of homeotic mutants and their analogs (Blochlinger et. al 1991) may eventually show that past models, such as gradual accumulation of changes that lead to adaptive peaks, are acceptable only for a few cases. I fully agree with Ernst Mayr's (1963) prophecy, "...I should think we are in for some surprises."

Figure 113. One of numerous vesicles composing the pygopod of lampyrid larvae.

Figure 114. Head of L. sanguineus larva, the bearer in figure 1.

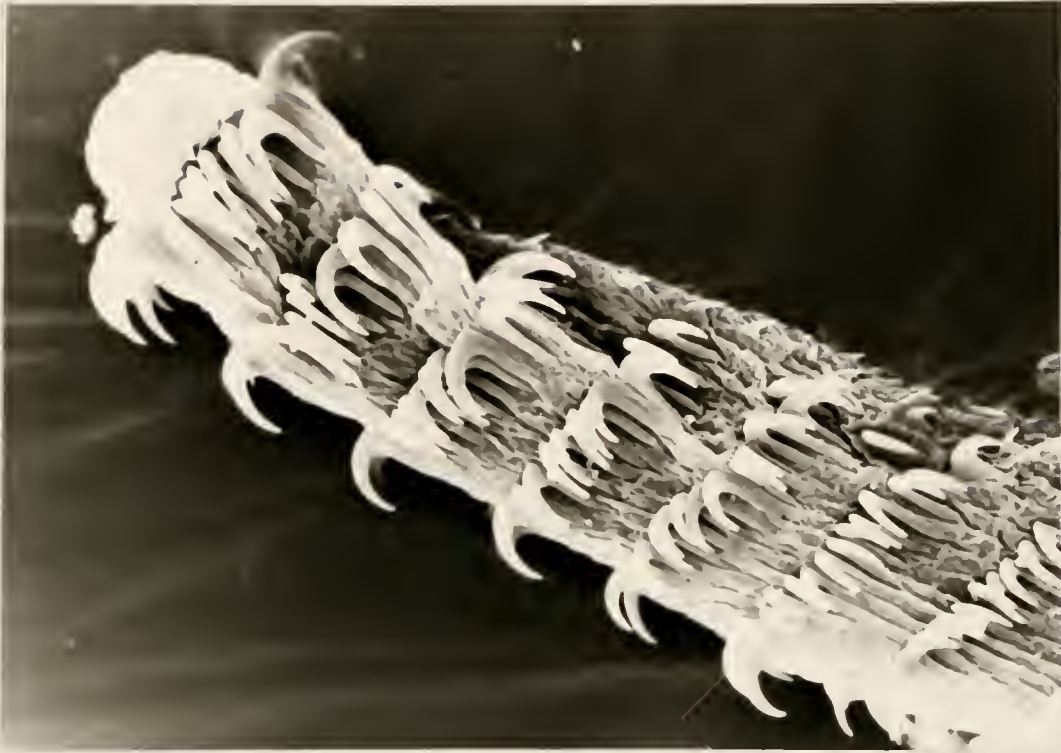


Figure 115. Abdominal apex of L. sanguineus larva. The pygopod is probably the protracted rectum, shown here in its retracted position.



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BIOGRAPHICAL SKETCH

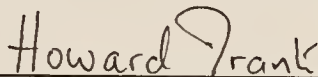
Two years after my birth in 1951 in Port Townsend, Washington, my parents moved permanently to the town of Pacoima, in the Los Angeles vicinity. I grew up there and, at about 16, developed a curiosity about insects from reading a very fine nature library put out by Audubon. I began making a collection immediately, using sewing pins and needles on cardboard. A few years later, after catching my first black witch and driving into a roadside rest area covered with hundreds of Xylorectes, there was no doubt that entomology was to be the choice for an academic vocation.

Darwin Tiemann (photos- Lopez 1987, Tiemann 1970) was certainly the most influential of my early acquaintances; together we general collected Arizona by day and hunted luminescent insects by night. I continued our studies on these insects after his death and am still doing so today.

I became a compulsive rock-kicker-over during my education at Alemany High School, Mission Hills, California. Education continued at Los Angeles Pierce College and was transferred to the University of California, Northridge, where I earned a BA in biology. I transferred my job with the US Post Office to Tucson, Arizona, bought my own house, and pursued my MS in entomology at the University there. My

Ph.D. was earned here at the Department of Entomology and Nematology, University of Florida, Gainesville.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Dr. J. Howard Frank, Chair
Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



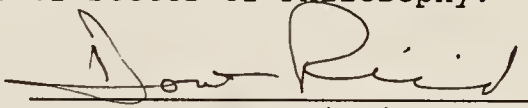
Dr. John Sivinski
Assistant Professor of
Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Dr. James E. Lloyd
Professor of Entomology and
Nematology

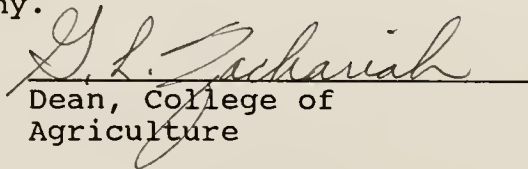
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Dr. Jonathan Reiskind
Associate Professor of Zoology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1991



Dean, College of
Agriculture

Dean, Graduate School

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